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CONTENTS

A STUDY OF THE BACTERIAL GENUS CHROMOBACTERIUM[1]

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INTRODUCTION

Pigment-forming bacteria have attracted attention from the earliest days of bacteriology. Prominent among them is the group of violet chromogenic bacteria commonly referred to as Bacterium violaceum, for which the generic name Chromobacterium is widely used.

Some authors include red and yellow chromogens in the genus, but in my opinion they should not be included, and the genus is therefore restricted here to violet chromogenic bacteria. The classification and nomenclature of these organisms is in bewildering confusion. Many different species are described in the literature, yet there appear to be only two good species. For this reason it has been necessary to include a considerable section upon the nomenclature. Little work has been done upon the relation of these bacteria to nonpigmented forms, and there is no good evidence for merging the genus with some other well-known genus. From the practical point of view therefore, the literature on violet bacteria has been reviewed, and since the distinction between shades of blue and violet may be a fine one, the blue pigmented bacteria have also been briefly treated. There are no authentic cultures of most of these, and many are now unrecognizable. A few violet chromogens which are clearly unrelated have been excluded, together with the purple bacteria of the families Thiorhodaceae and Athiorhodaceae, which are photosynthetic and chemosynthetic autotrophs which seldom grow on ordinary bacteriological media. Salient points on other well-known pigmented bacteria have been mentioned for comparison. In the historical section and in Appendix II the organisms have been numbered for ease of reference.

The working definition of Chromobacterium adopted here is: Gram-negative, aerobic bacilli which grow on the usual peptone media, motile, which produce a violet pigment (violacein) which is soluble in alcohol but not in chloroform or water. I shall follow the precedent of Buchanan (1955) in using chromobacteria as a vernacular name for members of the genus Chromobacterium, and if these names are in parenthesis this will indicate that the organisms referred to have in the past been placed in this genus, but should properly be excluded from it. However, names with a citation, e.g. Chromobacterium iodinum Davis, carry no implication as to the correctness of the name.

[1] Being the substance of a dissertation for the degree of Doctor of Medicine, University of Cambridge 1958 (Sneath, 1958).

The genus has not been thoroughly reviewed before. For this reason a very full bibliography has been compiled and the literature, which contains many scattered and incidental observations on these organisms, has been exhaustively reviewed. There is no full bibliography already published; of most value are those of Thiry (1900), Godfrin (1934), and Breed, Murray and Hitchens (1948). The most extensive early work on the genus is that of Bampton (1913), of Calderini (1925), and of Cruess-Callaghan and Gorman (1935). Strains of Chromobacterium were first reported to be pathogenic by Woolley (1904; 1905). The terminology for cultural appearances is that of Wilson and Miles (1946, Vol. 1, pp. 364-72).

Recent work has shown that there are two main groups of chromobacteria, a group growing best at 30° to 37° (Chromobacterium violaceum) and a group growing best at 20° to 30° (Chromobacterium lividum). They are also referred to here as the mesophils and the psychrophils respectively (the term psychrophil may be a misnomer as true psychrophilic bacteria seem to be rare, —see Ingraham, 1958—but it is the only convenient term available).

CHAPTER I

THE HISTORY OF THE GENUS

Early work on the genus

The first account of a violet chromogen of this group is that of Bacteridium violaceum of Schroeter (1872) (No. 123), though one may have been observed earlier by Lecoq de Boisbaudran (1882) (No. 75). Bergonzini (1880) described another strain which he called Cromobacterium [sic] violaceum (No. 124), and this has generally been taken as the type species of the genus. In the next few years other strains were described by Zopf (1883, p. 68) (No. 115), Hueppe (1884) (No. 141), Trelease (1885) (No. 125), Flügge (1886 p. 291) (No. 117), Eisenberg (1886 Tab. 2) (No. 83) and others. Kruckenberg (1881) described a blue discoloration of damp fibrin, and although he did not study the bacteriology, the solubility and the absorption spectrum of the pigment were similar to those of violacein.

Plagge and Proskauer (1887) first distinguished between organisms of the groups now called the mesophils and the psychrophils on the basis of gelatin liquefaction. de Lagerheim (1891), Sternberg (1893), and Fuller and Johnson (1899) recognized several species, but their differential features were trivial ones. The best early account is that of Lehmann and Neumann (1899, Vol. 2, pp. 262-265). With considerable restraint they recognized only two main types, Bacterium violaceum (No. 78) and Bacillus membranaceus amethystinus of Eisenberg (No. 100). Despite some discrepancies these types can be recognized as the mesophilic and psychrophilic groups respectively.

Thiry (1900, p. 118) in a classic thesis, divided the blue and violet chromogenic bacteria into six groups, the Micrococcus cyaneus group, the indigo bacteria, the violet bacteria (which is Chromobacterium) and three groups for the organisms Bacillus cyaneo-fuscus, Bacillus polychromogenes and Actinomyces violaceus. This scheme was sound and scholarly, and can be applied at the present day.

Bampton (1913) divided his strains of violet bacteria into Bacillus violaceus (No.108) and Bacillus membranaceus amethystinus (No.102) upon the following characteristics:

Characteristic	B. violaceus	B. membranaceus amethystinus
Potato slope	No growth	Good growth
Pellicle on broth	Scanty or absent	Thick
Rate of growth at 22°	Moderate	Slow
Metachromatic granules	Abundant	Scanty
Growth on gelatin	Not membranous	Membranous
Survival on heating at 45-47.5° for 30 minutes	Killed	Survive

The two species also formed different antigenic groups. It seems most likely from Bampton's descriptions that both of his groups were psychrophilic, and that his division was between strains giving membranous and nonmembranous growth, which, as shown by Corpe (1953), appear to be dissociants which can arise in the laboratory. I have not been able to obtain a clear-cut division of the genus by means of Bampton's tests.

Early workers were content to leave these organisms in the same genus as most of the other Gram-negative bacilli, usually Bacterium or Bacillus. Buchanan (1918, p. 52) recognized the genus Chromobacterium, with the type species Chromobacterium violaceum Bergonzini (No.124). Since then this generic name has been almost universally used. Buchanan placed the genus in the subtribe Bacteriinae of the tribe Bacterieae. Winslow, Broadhurst, Buchanan, Krumwiede, Rogers and Smith (1920 p. 209) placed it in a tribe Chromobactereae together with Erythrobacillus Fortineau 1904, p. 14 (= Serratia Bizio 1823) in the family Bacteriaceae. In the first edition of Bergey's Manual (Bergey, Harrison, Breed, Hammer, and Huntoon, 1923 p. 85) the same arrangement was adopted, but the tribe contained in addition the genera Flavobacterium and Pseudomonas. However the genus Chromobacterium (ibid. pp. 117-122) was a heterogeneous taxon, as it contained the unrelated organisms Bacillus caeruleus Voges 1893 (No.48), Bacterium visco-fucatum Harrison and Barlow 1905 (No.24) and Bacillus coeruleus Smith 1887 (No.35). The species which clearly belong to the genus were Chromobacterium violaceum Bergonzini (No.124), C. bamptonii (No.92), and C. membranaceum (No.99) based on two of the strains of Bampton (1913), C. lividum (No.94) based on the strain of Plagge and Proskauer (1887), C. janthinum (Zopf) Holland (No.115) and C. amethystium [sic] based on Bacillus membranaceus amethystinus of Eisenberg (1891) (No. 100). There were only minor modifications of this arrangement in subsequent editions of the Manual until the 5th edition.

Calderini (1925) studied forty strains of violet bacteria from Northern Italy. He considered that there were five groups of violet bacteria already described, the Bacillus violaceus group, the Bacillus amethystinus group, the Bacillus indigoferus group, the Micrococcus violaceus group, and the Bacillus polychromogenes group. He had strains of the first

three groups, and added two more groups which he did not name. Group VI were polar flagellate bacilli which produced a green fluorescent pigment upon nutrient agar and a violet pigment upon potato. Group VII were very similar to Bacillus amethystinus. Unfortunately Calderini's descriptions were fragmentary. The most interesting part of his work is that on the Group VI strains which may have been strains intermediate between Chromobacterium and Pseudomonas, and perhaps represent a new species; they do not seem to have ever been re-isolated. The other groups probably belong to Chromobacterium and to the "indigo" bacteria.

Enderlein (1925 p. 280) assigns the violet bacteria to the genus Bacterium, and Pribram (1933, pp. 3, 4, 49) assigns them to Pseudomonas. Topley and Wilson (1929, Vol. 1, p. 398) include in Chromobacterium the red chromogens of the "Bacillus prodigiosus" group (Serratia) and various yellow chromogens: it is a convenient but very heterogeneous taxon.

Cruess-Callaghan and Gorman (1935) made a careful study of the genus. They recognized three species upon the differential features shown below.

Features	B. violaceum	B. janthinum	B. membranaceum amethystinum
Flagella	Polar	Peritrichous	Polar, sometimes a few peritrichous
Acid from glucose	+	+	- or slight
Acid from maltose	+	-	- or slight
Acid from sucrose	-	-	- or slight
Nitrate reduction	+	- or slight	+
Growth on potato	Absent or slight	Moderate	Abundant
Growth at 37°	-	+	-
Growth at 2-4°	Slight	-	+

Cruess-Callaghan and Gorman did not give any reasons for the choice of names, and most of their tests have proved unreliable in separating the species. Bacterium janthinum consisted of mesophilic strains and their strains of B. membranaceum amethystinum were psychrophils, but the identity of the strains which they called Bacterium violaceum is uncertain, and it is possible that some were mesophils and some were psychrophils. Their scheme was adopted in the 5th and 6th editions of Bergey's Manual (Bergey, Breed, Murray and Hitchens 1919, pp. 92-94; Breed, Murray and Hitchens 1948, pp. 231-234). Most subsequent authors have found the descriptions inadequate or confusing, and have generally

called their strains Chromobacterium violaceum whether they were mesophils or psychrophils.

Conn (1938, p. 321) established the family Rhizobiaceae for the genus Rhizobium and other bacteria including Chromobacterium which were nonsporing rods, often pleomorphic, predominantly Gram-negative, and showed only a few flagella on each rod. He and his co-workers (Conn and Wolfe 1938a, 1938b; Conn, Wolfe and Ford 1940) noted the difficulty in distinguishing between the genera Rhizobium, Achromobacter, Alkaligenes and several soil bacteria and plant pathogens, and noted that these organisms seldom produce appreciable acid from carbohydrates as they oxidize glucose completely to carbon dioxide and water (see Sguros and Hartsell, 1952). They generally show "degenerate peritrichous flagellation," that is there are only a few flagella on each rod, often only one, and they may arise with equal frequency from the poles or the sides of the rods. They observed (Conn, Wolfe and Ford 1940; Conn and Elrod 1947) that strains of Chromobacterium showed similar flagellation, and in other respects were not dissimilar to Rhizobium, and the genus Chromobacterium was consequently placed in the family Rhizobiaceae in the 5th, 6th, and 7th editions of Bergey's Manual. In the 7th edition (Breed, Murray and Smith, 1957) the family includes besides Rhizobium and Chromobacterium the genus Agrobacterium proposed by Conn (1942) for the crown-gall bacillus A. tumefaciens and similar forms. It has been shown by Leifson (1956b) and Sneath (1956c) that the type of flagellation in Chromobacterium is of a new type; the cells may possess both polar and peritrichous (that is, lateral) flagella. This also vitiates the proposal of Tešić to place those pigmented rods which have polar flagella in a genus Chromopseudomonas (see Tešić, 1957). Also some strains, most mesophils in fact, produce quite large amounts of acid from glucose. These observations make it doubtful whether the genus is very closely related to Rhizobium. In the 7th edition of Bergey's Manual the species of Chromobacterium are listed as Chromobacterium violaceum (Schroeter) Bergonzini (the type species), C. janthinum (Zopf) Holland (No. 115), C. amethystinum (Chester) Holland (No. 100) and C. marismortui Elazari-Volcani (No. 64). The citation of the first should be Chromobacterium violaceum Bergonzini (No. 124). All the descriptions are inadequate and the names are confusing (Anonymous, 1958). C. janthinum is the mesophilic species properly called C. violaceum. C. amethystinum is the psychrophilic species properly called C. lividum. C. marismortui is very doubtfully a member of the genus. The Chromobacterium violaceum of the Manual appears to be founded mainly upon nongelatinous variants of C. lividum.

Godfrin (1934) monographed the blue and violet bacteria and gave an extensive bibliography, but contributed little new information. His work is in the main an uncritical compilation of the literature. Tobie (1945) suggested that the chemical nature of the pigment should be of major importance in classifying the chromogenic bacteria. He excluded Chromobacterium from Pseudomonas on the grounds that its pigment is not a phenazine derivative and while this step is a sound one, a number of authors have included in Pseudomonas certain bacteria which are otherwise very unlike pseudomonads on the grounds that their pigments are phenazine derivatives. Gilman (1953), for instance, suggests that the

organisms Chromobacterium viscosum Grimes (No. 25) and C. iodinum Davis (No. 61) should be transferred to Pseudomonas on these grounds. Gilman made a valuable contribution by examining the absorption spectra of the pigments of all the strains he could obtain which were assigned to the genus Chromobacterium. Besides the two mentioned above there were two other organisms, C. chocolatum (No. 50) and C. orangium (No. 51) which clearly did not belong to the genus. He studied four strains of Chromobacterium proper, all of them mesophils. These were the strains here called LW, SH, RT, and AM. The last mentioned was labelled C. amethystinum, though it is not a psychrophil, but in fact the psychrophils produce the same pigment (violacein) as the mesophils (Sneath, 1956b). Unfortunately Gilman's strain of C. ianthinum was mislabelled: it is the strain here called TI (No. 59) and does not belong to the genus. Strain TI produces a different pigment which is not violacein. The extensive work of Christopher and McCleskey (1941) and of Hans and Bicknell (1953) has unfortunately not been published in detail (McCleskey, personal communication; Hans, personal communication).

Chromobacterium was included with Serratia and Flavobacterium in a tribe Chromobacterieae of the family Bacteriaceae by Hauduroy, Ehringer, Urbain, Guillot, and Magrou (1937, pp. 49, 88). Magrou and Prévot (1948, p. 105) placed it in the tribe Chromobactereae (ascribed to Prévot, 1948, p. 16) with Serratia, Flavobacterium, and Protaminobacter in the family Pseudomonadaceae, recognizing three species, C. violaceum, C. ianthinum, and C. amethystinum. Brisou (1957) adds the genera Xanthomonas and Empedobacter gen. nov. to this tribe. These arrangements are open to the objection that the organisms are grouped together simply because they produce pigment. The genus is not explicitly classified by Orla Jensen (1909), Castellani and Chalmers (1919), Pringsheim (1923), Rahn (1929, 1937), Pribram (1929), Janke (1930), or Kluyver and van Niel (1936). Copeland (1956, pp. 21, 22) places Chromobacterium together with Serratia, Flavobacterium, Cellulomonas, and Achromobacter in the family which he calls Achromobacteriaceae, and which also includes the organisms of the coli-typhoid group. This family is included in the class Schizophyta of the kingdom Mychota.

Recent work on the genus

Corpe (1951) described a technique of isolating strains of Chromobacterium from soil which has proved of great use to subsequent workers (see Chapter IV). The strains which are recovered appear to be always psychrophils and it is still uncertain whether mesophils can be isolated by this method. Later Corpe (1953) observed that psychrophils which produce a tough membranous or "gelatinous" growth may dissociate into nongelatinous variants. He compared a number of strains isolated from soil with strains from other sources (Corpe, 1954) and noted that gelatinous strains were commoner among the soil strains. Three of his strains were mesophils (probably the same as the strains called here LW, RT, and SH). The other twenty-four were psychrophils, although two of them grew slightly at 37°. He found rather variable results with carbohydrate tests and did not attempt to separate the genus into species.

He noted that bubbles of gas were often formed in nitrate media, most commonly by soil strains.

Sippel, Medina and Atwood (1954) and Sippel (1955, pp. 41, 42, 59) made a brief study of mesophilic strains, particularly those isolated from cases of infection. Morris (1954) has studied strains isolated from soil in Trinidad. She was not able to separate them into species on the criteria given in the 6th edition of Bergey's Manual, but it is clear that her strains were mesophils, unlike the soil strains isolated by Corpe.

Leifson (1956a, 1956b) has made a detailed study of the genus, and his results are in close agreement with my own. He found that the strains fell into three groups:

Group 1. Sixteen strains which grew at 37° and fermented carbohydrates anaerobically, attacking glucose, fructose, mannose, and inulin, but not arabinose, xylose, maltose, mannitol or sorbitol. They liquefied gelatin rapidly. He proposed the name Chromobacterium manilae nov. comb. (see No. 81 in Appendix II).

Group 2. Six strains similar to Group 1 but unable to ferment carbohydrates anaerobically; they oxidized glucose and fructose aerobically, but no other carbohydrates. He proposed the name Chromobacterium laurentium (Migula, 1900) nov. comb. (see No. 80 in Appendix II).

Group 3. Six strains which did not grow at 37° and only attacked carbohydrates aerobically. They oxidized glucose, fructose, mannose, arabinose, xylose, maltose, mannitol, and sorbitol, liquefied gelatin slowly or not at all, and utilized citrate for growth. He proposed the name Chromobacterium violaceum (Schroeter) Bergonzini (see No. 109 in Appendix II).

He described the mixed form of polar and peritrichous flagellation and noted that the wavelength of the flagella tended to differ in the groups. He divided Group 1 on the basis of citrate utilization and slight differences in flagella into Subgroup 1a which did not utilize citrate promptly and Subgroup 1b which did utilize citrate promptly. All recently isolated pathogenic strains belonged to Group 1a. Leifson did not name the subgroups, and did not establish any type strains. His nomenclature is not wholly satisfactory, since it is doubtful if Group 2 agrees with the nomenclatural type Bacillus violaceus laurentius (No. 87) and other epithets have priority over 'manilae'. The name for Group 3 is ambiguous since it may mean Chromococcus violaceus (Schroeter) Bergonzini or Chromobacterium violaceum Bergonzini (see the following section on the history of the generic name), and it is very doubtful if either organism is recognizable as to its species. I am not in agreement in separating Group 2 from Group 1 as a distinct species at present, as I feel that the former may perhaps be loss-variants of Group 1 strains. Subgroups 1a and 1b may merit recognition as varieties, but it would seem superfluous to give them distinct names at present.

Eltinge (1956) proposed that the genus should be divided into three groups on the basis of tests with nitrate—strains which in some media can completely destroy all nitrate and nitrite, strains which reduce

nitrate but do not destroy all the nitrite and strains which do not attack nitrate at all. Unfortunately she does not give precise technical details nor does she say which test is the critical one in the event of disagreement between them. In my hands the results in her media are often conflicting. She has lately reviewed the recent work on the genus and proposed a nomenclature (Eltinge, 1957). In view of the proposals made to the Judicial Commission of the International Committee of Bacteriological Nomenclature (Sneath, 1956a) she gave alternative names to cover the possibility that the type species may be either a mesophil or a psychrophil. The type species has since been designated as a mesophil (Judicial Commission, 1958b).

Her classification may be summarized as follows (the names and epithets are those obtaining now that the type species is designated as a mesophil).

Genus Chromobacterium

Bacteria producing a violet pigment soluble in ethanol but not in chloroform or water.

I. Strains reducing nitrate completely beyond nitrite, oxidizing glucose, maltose and usually xylose and arabinose, and liquefying gelatin slowly. They usually grow at 4° but usually not at 37°.

Species 1. Chromobacterium lividum (Eisenberg 1891) Holland 1920.

A. Strains growing at 4° but not at 37°.
Subspecies 1a. C. lividum var. lividum var. nov.

B. Strains growing at 37° but not at 4°.
Subspecies 1b. C. lividum var. mesophilum var. nov.

II. Strains which reduce nitrate only to nitrite or do not reduce it at all, which oxidize glucose and often fructose but not maltose, and which may ferment glucose, sucrose, fructose, and arabinose, and liquefy gelatin rapidly. They grow at 37° and sometimes at 42° but not at 4°.

Species 2. Chromobacterium violaceum Bergonzini 1880.

A. Strains which ferment carbohydrates (i.e. anaerobically).
(i) Strains which reduce nitrate.
Subspecies 2a. C violaceum var. violaceum var. nov.
(ii) Strains which do not reduce nitrate.
Subspecies 2b. C. violaceum var. anitritum var. nov.

B. Strains which do not ferment carbohydrates. Nitrates are reduced to nitrites.
Subspecies 2c. C. violaceum var. purpureum var. nov.

The names which were proposed in the event of the type species being a psychrophil were:

Species 1. Chromobacterium violaceum (Schroeter) Bergonzini.
Subspecies 1a. C. violaceum var. violaceum var. nov.
Subspecies 1b. C. violaceum var. mesophilum var. nov.

Species 2. Chromobacterium janthinum (Zopf) Holland.
Subspecies 2a. C. janthinum var. janthinum var. nov.
Subspecies 2b. C. janthinum var. anitritum var. nov.
Subspecies 2c. C. janthinum var. purpureum var. nov.

In general, Species 1 is the psychrophilic group and Species 2 is the mesophilic group, although the identity of Subspecies 1b is doubtful, since I have been unable to get these strains to grow at 37°. The proposals on nomenclature do not contain adequate arguments for adopting them and the original literature is not cited. The varietal names are legitimate but, I feel, redundant, since one could distinguish a vast number of such varieties by using additional tests, and even with her tests I have observed other variants (e.g. loss of ability to reduce nitrate in a strain of Subspecies 2c). The varietal epithet 'purpureum' may possibly be a later homonym of 'laurentium' (see Leifson, 1956b). The citation Chromobacterium violaceum (Schroeter) Bergonzini is incorrect. Eltinge does not establish type strains for these varieties. She agrees with the proposal that the type species should be a mesophil (Sneath, 1956a) and agrees that strain MK (National Collection of Type Cultures No. 9757, American Type Culture Collection No. 12472) is suitable as the neotype.

The history of the generic name Chromobacterium

The name was first used by Bergonzini (1879) in the sense of a subgenus. He followed the classification of Cohn (1872) and subdivided Cohn's genera if they contained pigmented species. Thus he divided Micrococcus into two groups (gruppi), Micrococcus for nonpigmented species and Cromococcus [sic] for pigmented species, and (Bergonzini 1879, p. 38) Bacterium was divided into two sections (sezioni). He wrote "Il Gen: Bacterium come il gen. Micrococcus puo venir diviso in due sezioni:

Bacteri incolori (Bacterium)
Bacteri colorati (Cromobacterium)"

The genus Bacillus was similarly divided into Bacillus and Cromobacillus. The genera Vibrio, Spirillum and Spirochaete were not divided as he knew of no pigmented species of these genera. Bergonzini did not establish any type species, but the species which he lists in the subgenera of interest here, are, in order:

Cromococcus (pp. 35-37)
C. prodigiosus (pro synon. Micrococcus prodigiosus Cohn)
C. luteus (pro synon. Micrococcus luteus Cohn)
C. candidus (pro synon. Micrococcus candidus Cohn)

C. fulvus (pro synon. Micrococcus fulvus Cohn)
C. aurantiacus (pro synon. Micrococcus aurantiacus Cohn)
C. cyaneus (pro synon. Micrococcus cyaneus Cohn)
C. violaceus (pro synon. Micrococcus violaceus Cohn syn. Bacteridium violaceum Schroeter 1872)

Cromobacterium (pp. 39-40)
C. syncyanum [sic] (pro synon. Bacterium syncyanum Schroeter, Vibrio syncyanus Ehrenberg)
C. aeruginosum (pro synon. Bacterium aeruginosum Schroeter)
C. brunneum (pro synon. Bacterium Brunneum Schroeter)
C. xanthinum (pro synon. Bacterium xantinum [sic] Schroeter, Vibrio synxanthus Ehrenberg)

Cromobacillus (p. 43)
C. ruber (pro synon. Bacillus ruber Cohn)

The importance of these names is because the rules of bacteriological nomenclature require that a genus must be founded on a type species and this type must be one of the species included in the genus when the generic name was first published (Rule 9(2)c2, International Code of Nomenclature of Bacteria and Viruses, 1958). In this instance Cromobacterium is an invalid generic or subgeneric name (the two are subject to the same Rules) for the violet bacteria, since none of the species listed are violet bacteria. The name may also be a synonym of Pseudomonas Migula, since the recognizable species appear to be pseudomonads, and Pseudomonas has been conserved with type species Pseudomonas aeruginosa (Schroeter 1872) Migula, 1900 (syn. Cromobacterium aeruginosum Bergonzini) (Anonymous 1951; Judicial Commission 1952). Indeed Enlows (1920, p. 74) designates Pseudomonas violacea as type species of Pseudomonas by monotypy, but this refers to Migula's use of the genus in 1894, when the only species mentioned, P. violacea is not validly cited (Migula 1894, p. 237). In 1895 Migula again used the name but P. violacea is the second organism listed, so the genus is not monotypic (Migula 1895, p. 29).

In 1880 Bergonzini discovered a new violet bacterium, and placed it in Cromobacterium under the name C. violaceum (Bergonzini 1880, p. 153). The spelling of the generic name is not a typographic error, being the conventional Italian transliteration from the Greek. It ranks as an orthographic variant of the usual and preferable form which was first used in a review of this second paper (Anonymous, 1881) and the emended spelling has generally been followed since (Grove 1884; de Toni and Trevisan 1889; Buchanan 1918). The exact date of Bergonzini's second paper is doubtful: the title page of the volume is dated 1881, but it may have been issued in parts during 1880 and another review (Zimmermann, 1880) appeared in Section 49/50 of the Botanische Centralblatt, 1880, though the day and month of issue are not given on the Sections. I have assumed that the correct date is 1880.

Buchanan (1918, p. 52) revived the name Chromobacterium with emended spelling and description, and circumscribed it so as to include virtually only the violet bacilli. He was not aware of Bergonzini's paper of 1879, and cited it as a full genus. His description is:

"Chromobacterium Rod-shaped bacteria without spores, aerobic, producing a violet chromoparous pigment soluble in alcohol but not in chloroform, motile or nonmotile, Gram stain variable. The type species is Chromobacterium violaceum Bergonzini."

Unfortunately Bergonzini's organism is, in my opinion, not recognizable as to species. This leaves the genus, already illegitimate, with an unrecognizable type species. The generic name Cromococcus would be available, since one of the species mentioned is Schroeter's organism Bacteridium violaceum. Cromobacillus is not available since the only species mentioned is not a violet bacterium. The name has also been used as a subgenus by Hansgirg (1885, p.265) with Cromobacillus sanguineus (Schroeter 1886, p.173) Hansgirg as the type by monotypy, which is also not a violet bacterium. However, the over-riding reason for retaining the name Chromobacterium is that as emended by Buchanan it has become so firmly fixed in bacteriology that to change the name would cause needless confusion. Proposals were therefore made (Sneath, 1956a) to the Judicial Commission of the International Committee on Bacteriological Nomenclature to validate the name by conservation, with unchanged but redefined type species and the Commission has now done this (Judicial Commission, 1958a; 1958b). Gieszczykiewicz (1939, p.21) changed the type species to C. prodigiosum but this is not permitted by the rules of nomenclature. The finer legalistic points have been fully dealt with elsewhere together with the reasons for considering the epithets violaceum and lividum as the correct ones for the two species which I recognize in the genus (Sneath, 1956a). The Opinion of the Judicial Commission (1958b) is given below.

Opinion 16. Conservation of the generic name Chromobacterium Bergonzini 1880 and designation of the type species and the neotype culture of the type species.

1. The generic name Chromobacterium Bergonzini 1879 is rejected and placed in the list of nomina generica rejicienda.

2. The generic name Chromobacterium Bergonzini 1880 is conserved and placed in the list of nomina generica conservanda.

3. The type species of the genus Chromobacterium Bergonzini 1880 is Chromobacterium violaceum Bergonzini 1880.

4. A neotype strain of Chromobacterium violaceum Bergonzini 1880 is designated and has been deposited in the American Type Culture Collection, Washington, D.C. (12472) and in the National Collection of Type Cultures, London (9757).

CHAPTER II

CHROMOGENESIS IN BACTERIA

Bacterial coloration of natural products has been a favorite subject for historical study in bacteriology. The history of "bleeding polenta" and other manifestations of the growth of "Bacillus prodigiosus" upon foodstuffs has been reviewed by Breed and Breed (1925) and Reid (1936; 1937). Blue, green or yellow discoloration of milk has long been known. There are few records of violet discoloration of natural materials, the most notable being violet coloration of egg-white (Bergonzini, 1880), of fibrin (Kruckenberg, 1881), of flour paste (Lecoq de Boisbaudran, 1882), of a pig's bladder (Zopf, 1883), of butter (Dehusses and Novel, 1939) and of wool (Seddon, 1937). Other instances are listed in the section on the distribution of Chromobacterium.

There are many types of bacterial pigment, and from a practical point of view a cimple classification of these is useful. Migula (1897, p.283 et seq.) and Buchanan and Fulmer (1928; 1930; Vol.1, p.115) base the divisions upon the solubility of the pigments, and a scheme of this kind is given below for convenience, listing some of the pigments which are treated at greater length later.

I. Insoluble in water
  (a) Soluble in chloroform e.g. prodigiosin from "Bacillus prodigiosus," iodinin from "Chromobacterium iodinum," carotenes.
  (b) Insoluble in chloroform
    (i) Soluble in alcohol e.g. violacein from Chromobacterium.
    (ii) Insoluble in alcohol e.g. indigoidine from the "indigo bacteria."

II. Soluble in water e.g. pyocyanin from Pseudomonas aeruginosa, viscosin from "Chromobacterium viscosum."

Beijerinck (1891a; 1891b; 1892) divided chromogenic bacteria into three groups: chromophores in which the pigment fulfils an obvious physiological purpose such as bacterial chlorophyll, chromopares in which it is a useless excretion product (e.g. pyocyanin) and parachromopares in which it is a useless excretion product but remains in the cells (e.g. prodigiosin). His terms may at times be convenient ones.

Chromogenic bacteria are known from many bacterial genera, well-known examples being Chromobacterium, Serratia, Flavobacterium, Micrococcus, Mycobacterium, Pseudomonas, and Actinomyces. The purple bacteria of the families Thiorhodaceae and Athiorhodaceae have been known for a long while (they were probably among organisms described by Kützing (1849, p.472), Perty (1852, p.174) and Rabenhorst (1864-1868, passim). They contain reddish bacteriopurpurins, which are carotenoids, and bacterial chlorophyll. Some cocci and mycobacteria also appear to contain carotenoids (Reid, 1937). Chromogenic strains occur in many taxa which are typically not pigmented. Examples are the rust-coloured strains of Lactobacillus (Breed and Pederson, 1938), yellow strains of Escherichia (Tittsler, 1939; Gilliland and Vaughn

1943), yellow and pink strains of Micrococcus tetragenus (Reimann and Eklund, 1941), green fluorescent strains of Azotobacter (Johnstone, 1955) and of Bacillus cereus (Smith, Gordon and Clark, 1952, p. 58) and chromogenic strains of Clostridium (McClung, 1943; Carbone and Venturelli, 1925). Such variants are nowadays not usually given specific names, but some authors find the temptation hard to resist, for example Malligo, Parr and Robins (1955) grouped the yellow strains of Escherichia into a species Escherichia aurescens.

There are also many bacteria which are not chromogenic upon ordinary bacteriological media, but produce pigment if certain substances are added. Thus Mycobacterium tuberculosis is reported to produce abundant yellow pigment in the presence of para-aminobenzoic acid (Mayer, 1944), and Gray (1928) reported several bacteria which produce indigo from indole. In a few instances pigment formed on ordinary media is known to be produced from a specific substance, such as the brown pigment of some strains of Bacillus subtilis, which is formed from tyrosine (Smith, Gordon and Clark, 1952, p. 30). In the majority of instances no such substance is known, and the pigment is probably produced not from a specific constituent of the medium but as a by-product of general metabolism. The physiological action of most bacterial pigments other than bacterial chlorophyll is unknown, and the evidence that they have any function is very conflicting. Recent work by Garibaldi and Neilands (1956) suggests that some may be involved in iron metabolism.

Red chromogenic bacteria

The red chromogens are of some interest because it is possible that the "Bacillus prodigiosus" group may be closely related to Chromobacterium. The correct name for "Bacillus prodigiosus" is, according to Breed and Breed (1924), Serratia marcescens Bizio. The most extensive work is that of Hefferan (1903; 1904; 1905). She divided them into five groups, the 'prodigiosus' group, the 'rubricus' group, the 'mycoides roseus' group, the 'lactis erythrogenes' group and the 'mesentericus ruber' group. The last are spore-forming rods which clearly belong to the genus Bacillus. Some red chromogens have been transferred to various genera (Breed and Breed, 1927; Breed, 1936), but apart from Serratia they are still poorly known. Lochhead (1943) placed the red halophilic bacteria in Pseudomonas and Anderson (1954) created the genus Halobacter for them. It has become clear that Serratia is closely related to the coliform organisms, and is commonly included in the family Enterobacteriaceae, for instance by Kauffmann (1949) and Breed et al., (1948, p. 479; 1957, p. 359).

CHAPTER III

SOURCE OF THE STRAINS AND CLINICAL MATERIAL

Strains

Thirty-eight strains of Chromobacterium have been fully studied. In addition a number of strains which have in the past been placed in this genus have also been fully studied. These are Chromobacterium viscosum Grimes 1927 (No. 25), Chromobacterium iodinum Davis 1939 (No. 61), and Chromobacterium ianthinum Gilman 1953 (No. 59). For purposes of comparison two strains of Pseudomonas aeruginosa (NCTC 2000 and NCTC 6749) and two strains of Serratia marcescens (NCTC 1377 and NCTC 9493) were also included in the main study. The protocols on all these bacteria have been kept together, but those strains which are not true chromobacteria are described separately in Appendix II. Sources of these forty-five strains are listed in Table 1 (taken from Sneath, 1956b). Since completing the main bulk of the work a number of other strains have been received and briefly examined. These are listed in Table 2, and the results have been summarized in later Sections where they are of interest.

Isolation of strains. Most of the strains studied were isolated by other workers. Two strains (BH and BN) were isolated by me from cases of infection, from cases 11 and 12 of Sneath et al. (1953), from pus and urine respectively, by plating onto blood agar. Four psychrophilic strains were isolated by me from soil by the technique of Corpe (1951) described in the next chapter. In many instances, although violet patches were seen on the rice grains, subcultures from these patches onto nutrient agar and blood agar were so overgrown by other organisms that no strains of Chromobacterium were isolated. Of nine soil samples seven gave violet patches when incubated at 24°, and several gave such patches when incubated at 2°, but no violet patches were seen when the samples were incubated at 30° or at 56°. Several other media were used to try and increase the percentage of successful isolations. Plates made from a medium consisting of 1% of mashed and strained boiled rice grains 1.5% of agar and 100 ml of tap water gave good growth of psychrophilic strains, but pigmentation was usually so poor that recognition was difficult. The addition to this medium of 25 μg/ml of L-tryptophan (suggested by the work of DeMoss and Happel (1955) on the biosynthesis of violacein) gave better pigmentation, and this medium was used for the isolation of the strains RU, GA, DA, and NC, by plating violet patches from the rice onto this medium and incubating for some days at 25°.

Methods of preservation. Strains of Chromobacterium do not live long in cultures and therefore all strains studied have been kept in freeze-dried form. Heavy suspensions from agar were emulsified in sterile horse serum containing 5% of glucose, freeze-dried and sealed in vacuo in ampoules. From these ampoules they were recovered and maintained during periods of some months as cultures in nutrient broth kept in the

dark at room temperature and subcultured every two weeks. At 4° such cultures frequently became sterile in two weeks. The strains were recovered from desiccates every few months to avoid continued subculture in broth. A few strains have also been stored as young broth cultures frozen at -78°. When strains were dried, the colonies were examined for dissociation, and the smooth and well-pigmented colonies were selected. By these methods the strains have been fairly stable, although slight loss of pigment, slight degrees of roughness, and a decrease of virulence are difficult to avoid.

Clinical material

The clinical material which I have studied consists of the following cases:

Case 1. Case 11 in Sneath et al. (1953), a case of urinary infection in man, from which strain BH was isolated.
Case 2. Case 12 in Sneath et al. (1953), a case of septicaemic infection in man, from which strain BN was isolated.
Case 3. Case 13 in Sneath et al. (1953), a case of bowel infection in man.
Case 4. Case 14 in Sneath et al. (1953), a case of bowel infection in man.

The case notes are included in Appendix I.

CHAPTER IV

HABITAT AND METHODS OF ISOLATION

Habitat

The great majority of strains of Chromobacterium have been isolated from fresh water. There are records of their isolation from fresh water by Macé (1887a), Imbeaux (1897, p.134), Macé and Imbeaux (1899), Bréville (1897, pp.95, 97, 99), Korn (1898), Miquel (1891, p.23), Pokrowsky (1891), Fuller and Johnson (1899), Bidet (1900, quoted by Miquel and Cambier, 1902, p.697), Matruchot (1900), Casagrandi (1912), Reiss (1912), Gamm (1936) and several discussed below. No doubt there are many more such records scattered throughout the literature. Whether these have been mesophils (i.e. C. violaceum) or psychrophils (i.e. C. lividum) is usually uncertain, but in the tropics several authors have isolated strains from water which were clearly mesophils (e.g. Lesslar, 1927; Martin, 1931; Minett, 1913; Audebaud et al., 1954; Gauducheau, 1907; Sneath et al. 1953; Sippel, 1955, p.64), as well as other authors mentioned previously.

Schattenberg and Harris (1942) made enquiries among bacteriologists and found that violet bacteria were not commonly seen; they were sometimes isolated from water, but rarely from foodstuffs or in medical bacteriology.

Table 1. Source of strains

Strain letters	NCTC No.	From whom received and date	Name or No. as received	Details of isolation where known		
				Investigator	Source	Country and Date
FH	9373	Whelan, 1952	Frazer's Hill	Whelan	Water	Malaya, 1952
MK	9757	Whelan, 1952	Mentekab	Whelan	Water	Malaya, 1952
LG	9374	Whelan, 1952	Lake Gardens	Whelan	Water	Malaya, 1952
BH	9696	--	--	Sneath	Human urine	Malaya, 1951
BN	9694	--	--	Sneath	Human fatal case	Malaya, 1952
AM	9370	BSC, 1952	C. membranaceum amethystinum	--	From NCTC in 1935	--
MW	9371	MWB, 1952	--	Prof. Thomas	Water	England, --
NT	7917	NCTC, 1952	NCTC 7917	Collins, FBA strain 2	Lake water	England, 1949
TV	--	HCC, 1952	C. violaceum	HCC	Forest soil	Trinidad, 1950
LW	8683	ATCC, 1952	ATCC 7461, Lewitus strain	--	--	-- --
SH	8684	ATCC, 1952	ATCC 6357, Shahan strain	Shahan	Human infection	Florida, USA, 1937
RT	8685	ATCC, 1952	ATCC 553, Rettger 4/22 strain	L.F. Rettger, Yale Univ.	--	--
SL	9695	Sippel, 1954	Sealy	Sippel	Swine infection	Georgia, USA, 1953
RV	9372	Sippel, 1954	Reeves	Sippel	Bovine infection	Georgia, USA, 1952
DK	9376	Darrasse, 1955	135	Darrasse	Human infection	Dakar, Africa, 1954
MH	9377	U.M., 1952	--	--	--	--
EA	--	Eltinge, 1955	60	Eltinge	Soil	New Hampshire, USA, 1953
EB	--	Eltinge, 1955	30	Eltinge	Dust	Massachusetts, USA, 1952
EC	4737	Eltinge, 1955	47 (ATCC 6915)	Prof. Kluyver strain L91	--	--

RU	--	--	--	Sneath	Soil	England, 1955
GA	--	--	--	Sneath	Soil	England, 1955
DA	--	--	--	Sneath	Soil	England, 1955
NC	--	--	--	Sneath	Soil	England, 1955
TA	--	HCC, 1955	C. violaceum	Morris, HCC	Water	Trinidad, 1955
TB	--	HCC, 1955	C. ianthinum		Institut Pasteur, Paris	
MA	--	MWB, 1955	Ch 25	MWB	Water (sand filter)	England, 1955
MB	--	MWB, 1955	Ch 27	MWB	Water (sand filter)	England, 1955
MC	--	MWB, 1955	Ch 28	MWB	Spring water	England, 1955
PT	--	Sippel, 1955	Porter	Sippel	Swine infection	Florida, USA, 1955
HA	--	Hans, 1955	H-4	Hans	Soil	Michigan, USA, 1952
HB	9796	Hans, 1955	H-24	Hans	Soil	Michigan, USA, 1952
HC	--	Hans, 1955	6-15	Hans	Soil	Michigan, USA, 1952
HD	--	Hans, 1955	6-20	Hans	Soil	Michigan, USA, 1952
HE	--	Hans, 1955	Traunstein	Hans	Soil	Germany, 1955
HF	--	Hans, 1955	Ulm	Hans	Soil	Germany, 1955
IN	--	Hans, 1955	Indiana X	Indiana Univ.	Soil	--
CA	--	Hans, 1955	Corpe	Corpe	Soil	USA --
PB	--	Hans, 1955	Institut Pasteur, Paris, 52227	--	Water	-- 1952
GR	2416	NCTC, 1955	NCTC 2416 (ATCC 6918) Corynebacterium viscosum	Grimes	Butter	Ireland, 1927
TE	9742	ATCC, 1955	ATCC 9897 Pseudomonas iodina	Davis	Milk	England, 1938
RE	--	NIRD, 1955	Chromobacterium iodinum Davis, NIRD 613	Davis	Milk	England, 1938
TI	--	HCC, 1952	C. ianthinum	HCC S11 (Morris and Roberts, 1959)	Forest soil	Trinidad, 1950

Abbreviations. The addresses of workers and of the institutes (indicated above by abbreviations) that provided strains are given in the Acknowledgements. Strains RU, GA, DA and NC were isolated by the method of Corpe (1951).

Table 2. Additional strains which have been briefly studied.

Strain letters	From whom received and date	Name or No. as received	Details of isolation
BHR/1	--	--	A rough variant of BH
BNR/1	--	--	A rough variant of BN
ED	Eltinge, 1957	E 41	From soil, Georgia, USA, by Eltinge
EF	Eltinge, 1957	E 91 (Corpe strain D3)	From soil, Kentucky, USA, by Corpe.
BC	Shewan, 1957	CHBC	Isolated from codfish stored on ice
BM	Whelan, 1957	--	Isolated by Whelan from brain of a monkey said to have died of rabies

Comments on the above strains

BHR/1 and BNR/1. These were obtained by growing the parental strains in broth containing BH and BN anti-O sera respectively. They were antigenically and colonially slightly rough and were of lowered virulence, but were otherwise identical with the parental strains.

ED and EF. These were sent as representatives of the variety 'mesophilum' of Eltinge, 1957. In my hands they would not grow at 37°, although slight growth was seen at 35°. They appear to be psychrophilic strains which are atypical in that they do not hydrolyze aesculin, and EF does not produce detectable acid from glucose even aerobically.

BC. This may have come from freshwater, as the fish were stored on ice. It is a typical psychrophil except for an unusual power of digesting chitin.

BM. This is a typical mesophil.

Rice (1938) studied the occurrence of what he called Chromobacterium lividum (the species is uncertain) in water from a creek. He noted that it was more frequent when the water was running freely, which coincided with drainage works and road-making in the watershed. In one year over 1% of the colonies on plates were violet chromogens, but in other years there were only one or two per 10,000 colonies. Buffle and Pongratz (1950) found Bacillus violaceum together with Bacillus fluorescens liquefaciens (presumably Pseudomonas fluorescens) in a bacterial zoogloea on Lake Geneva. Shortly afterwards there was a great development of the diatom Melosira.

Strains have been isolated from both stagnant water and from streams and rivers. Grimes (1930) found a strain from an artesian well 66 feet deep. Poncet (1895, p. 51, Pl. 9, Photo 13) reported them from a mineral spring, though his description is not convincing. Kohn (1906) noticed that in stored water Chromobacterium appeared after seven to ten weeks, succeeding a flora of pseudomonads, yeasts, and micrococci, and being succeeded by "Bacillus ruber" and Sarcina flava. None of the nutrients which he added to the water increased the number of Chromobacterium.

There is no consensus of opinion on the significance of Chromobacterium in water supplies. Some (e.g. Buttiaux, 1951, p. 48) thought it a sign of putrefying organic matter. Others (e.g. Imbeaux, 1897, pp. 123, 134; Bréville, 1897, pp. 51, 62) considered that it indicated faecal pollution. Gray (1951) thought that it was characteristic of very cold waters, but Miquel (1891, p. 23) found that it quickly died out in water at 0°. It is, of course, possible that the significance of mesophils and psychrophils in water is somewhat different.

A number of investigators have isolated strains from soil (Thiry, 1900, p. 108; Janke and Woznak, 1934; Starc, 1941; Veldkamp, 1955). Macé (1888a, 1889, p. 688) found it in soil taken from depths of 2 to 3.2 metres. Calderini (1925) made a valuable contribution to the biology of the genus by showing that strains occurred frequently in soil and were most common in water after heavy rain; he therefore considered that their presence in water was an index of soil pollution. Corpe (1951) was able to isolate strains from 90% of soil samples by a technique described later; he estimated that their numbers are usually 100 to 10,000 per gram of soil. He also isolated it from water, but noted that strains from water were less commonly gelatinous and were less active denitrifiers, and thought that water might be the natural habitat of some strains (Corpe, 1954). Most of these isolates from soil were probably psychrophils, but Morris (1954) frequently obtained mesophilic strains from soil in Trinidad by direct plating. She found them most common in moist soils which were rich in humus. The natural habitat of the genus therefore seems to be soil and perhaps also water, though it is possible that they are not true members of the water flora, but simply survive longer in water than most other soil bacteria.

The rôle of the genus in nature is obscure. These bacteria may be important as denitrifying organisms (Corpe, 1954). They are possibly protected by the antibiotic action of their pigment against competing bacteria and predatory protozoa. The strains reported by Bromfield and Skerman (1950) to be involved in oxidation of manganese in the soil were pink chromogens (Bromfield, personal communication).

There are records of isolation of strains of Chromobacterium from many other sources, though probably they were originally derived from water or soil. Minett (1913) found mesophilic strains in milk in British Guiana, and Reilly and Pyne (1927) studied a strain from milk. Chromobacterium does not seem to give rise to outbreaks of "violet milk" (as was noted by Hueppe, 1884) and Rühm (1910) evidently confused it with Pseudomonas syncyanea (No. 33). Artault (1893) and Zörkendörfer (1893) isolated strains from hen's eggs. Deshusses and Novel (1939) observed a psychrophilic strain causing violet patches on butter. Pigulevskii and Kharik (1928) isolated two violet organisms from rancid butter, but did

not describe them. Strains (probably psychrophils) were isolated from air or from dust by de Lagerheim (1891) and Germano (1892). Bujwid (1887, 1888) found strains in hailstones, but they were probably derived from ponds etc. by storm winds. Strains have been isolated also from bread (Matruchot, 1900), snow (Schmelck, 1888), sewage effluent (Jordan, 1890), and sand from water filtration beds (Boyce and Hill, 1900). Organisms which may have been strains of Chromobacterium have been isolated from fibrin (Krukenberg, 1881), starch paste (Lecoq de Boisbaudran, 1882), pressed yeast (Marpmann, 1896) and fish roe (Seiser, 1925). Kisitani and Sumiyosi (1939) commonly found strains on the gills of the freshwater Venus mussel, Meretrix meretrix, and report that Hasioka isolated strains from the crystalline style of this mollusc: probably the organism was filtered from the water.

Violet discoloration of wool on Australian sheep may be due to Chromobacterium (Seddon, 1937). The responsible organism was not isolated from the wool, but the violet pigment behaved very like violacein and Seddon was able to produce the phenomenon by inoculating fleeces with cultures of a strain of Chromobacterium. The discoloration only appears where the fleece is thick and constantly wet and appe most often as small patches on the back and withers, associated with "fleece rot." The violet colour forms a band near the base of the hairs, and consists of pigment granules which can be washed off, leaving the wool white. It is therefore not of great economic importance, unlike the commoner green discoloration due to Pseudomonas aeruginosa. This latter organism can also stain wool red or blue according to Fraser and Mulcock (1956) but apparently not violet. Seddon also described lilac discoloration caused by an unnamed pseudomonad which produces a green fluorescent pigment and also purple crystals of pigment, and he also thought that "Chromobacterium coeruleum" could cause blue discoloration of wool.

A number of instances are known of infection in mammals by strains of Chromobacterium, which are listed in Chapter XIX. They do not appear to cause infections of insects (Steinhaus, 1941; 1946) nor of plants (Elliott, 1951) unless the obscure instance described by Thiry (1900, p.108) refers to this genus. Thiry mentions the isolation of a violet bacillus from a wound in the plant Tricholoma, but gives few details.

The geographical distribution of the genus is world-wide. Mesophilic strains appear to be commoner in the tropics and psychrophilic strains in temperate climates but our knowledge on this point is still small. There seems to be no record in the literature of a true Chromobacterium from a marine environment (the references given by ZoBell (1946) are not to members of the genus sensu stricto).

Strains of Chromobacterium have been used by Fränkel and Piefke (1890) and by Bruns (1948) as a tracer organism for testing water filtration plant and similar purposes.

Methods of Isolation

The usual media suffice for the isolation of strains of Chromobacterium from infected animals, though blood agar is more suitable than plain agar because small inocula may not grow on the latter (see Chapter VIII).

Strains may be isolated from water on gelatin or nutrient agar plates. Pour-plates are less suitable since deep colonies may not become pigmented because of lack of oxygen. The temperature of incubation is of course important, but both mesophils and psychrophils can grow at 20° to 25°, so for routine purposes this temperature range is best. It is, however, worth noting that Thomas and Thomas (1947) found that about 6% of the colonies from farm water inoculated on agar media and incubated at 3-5° for four weeks were colonies of Chromobacterium, and it is likely that low temperatures are most suitable for the isolation of psychrophils.

The methods mentioned above do not succeed if there are large numbers of other organisms present. The enrichment technique generally used at present is that of Corpe (1951). A few grains of soil or a few millilitres of water are placed in a Petri dish and sterile distilled water is added to give a total volume of 10 to 25 ml. About fifty grains of sterilized polished rice are sprinkled on the water, and the plates are closed and incubated at 20° to 25° for five to ten days. Violet patches form on the rice and strains of Chromobacterium can be isolated from the patches by plating onto nutrient agar and incubating at room temperature. As mentioned in Chapter III, a rice-tryptophan agar is also useful and incubation at 1-4° for some weeks may be advantageous.

Beijerinck (1916) used several enrichment methods which have been generally overlooked. His first method was to inoculate soil or water onto plates composed of 1 to 2% of dried blood fibrin, 3% of agar and 0.02% of potassium chloride, and to incubate them at 22 to 25°. Egg white could be substituted for the fibrin. He also observed strains growing on wheat gluten steeped in water, and devised a second method in which washed barley grains were kept moist by placing on filter paper whose edge was in a bowl of water. Violet patches formed on the grains as in Corpe's method. A third method (attributed to Jacobson) was to place a piece of white bread near a dripping water-tap so that it was kept moist by being splashed with spray and to subculture from any violet patches which appeared.

There is little information on whether these methods cause enrichment only of psychrophils, or of mesophils, or of both, but as far as one can tell, it is only psychrophilic strains which are isolated by Corpe's technique. Dr. R. S. Wolfe confirms this, and recommends 0.025% yeast extract in watery agar for the subculture from the rice.

CHAPTER V

MORPHOLOGY

Size and shape

The majority of authors have found that organisms of the genus Chromobacterium are rod-shaped, measuring 0.5 to 1 μ broad and 1 to 5 μ long. A few have noted longer forms up to 15 μ long (e.g. Bampton, 1913). Some have reported them to be less than 0.5 μ in breadth (Cunningham and Raghavachari, 1924; Cruess-Callaghan and Gorman, 1935). In general mesophils (i.e. C. violaceum) are smaller than psychrophils (i.e.

C. lividum). The ends are rounded and the rods may be slightly curved (Woolley, 1904, 1905; Minett, 1913; Gauducheau, 1907). They are arranged singly with a few pairs and occasionally chains of five to ten organisms are seen. They may show slight pleomorphism. They have never been reported as having definite capsules, though Corpe (1954) noted intercellular slime and many strains (the gelatinous psychrophils) form a tough membrane or zoogloea in culture.

The size and shape of the 45 strains was studied in films stained with Löffler's methylene blue, from nutrient agar cultures incubated at 25° for 18 hours and for 4 days. Size was measured with an eyepiece micrometer and oil-immersion objective, and the values are only approximate. The findings at 18 hours are shown in Protocol 1 (Appendix I), and may be summarized as follows:

All strains were rods with rounded ends. The mesophilic organisms were smaller than the psychrophilic organisms, the mean size of mesophils being about 0.75 x 1.9μ, and of psychrophils about 0.95 x 3.7μ. However one mesophil (strain AM) was larger, about 1.0 x 5μ, and one psychrophil (strain MB) was small, about 0.8 x 2μ. Some mesophils showed many coccobacillary forms. The longer rods were occasionally distinctly curved in both mesophils and psychrophils, and there was very little pleomorphism. The rods were arranged singly or in pairs as a rule, but a few psychrophils showed short chains of five or more members. After 4 days growth there was less difference in the size of the two kinds of organism: the mesophils averaged about 0.75 x 2 μ and the psychrophils about 0.85 x 2.5μ. There was still very little pleomorphism after 4 days, and the other features also remained almost the same.

Flagella

The great majority of authors have found strains of Chromobacterium to be motile. A few (Eisenberg, 1891, p. 421; van der Sleen, 1894) have found nonmotile strains, but Ramchandani (1930) and Martin (1931) obtained motile forms by subculture of initially nonmotile strains in semi-solid agar. Nonmotile strains appear to be rare, since Bampton (1913), Calderini (1925), Grimes (1930), Cruess-Callaghan and Gorman (1935), and Corpe (1953, 1954) found none in studying an aggregate of about 100 strains. Ward (1898) believed they ceased to move in the dark, but how he observed this is not clear.

There has been considerable confusion on the flagellar arrangement in these bacteria. Migula (1895; 1900, pp. 939, 941, 942, 943, Pl. XIII, fig. 6), Wright (1895), Kisitani and Sumiyosi (1939), Corpe (1953), Waeldele (1938), and Sippel et al. (1954) found a single polar flagellum on their strains. Mehta (1925), Wilson and Miles (1946, Vol. 1, p. 634), Cunningham and Raghavachari (1924) and Deshusses and Novel (1939) reported peritrichous flagella. Other authors reported various mixed forms of flagellation. Pepplar (1901) reported 1-6 flagella, Bampton (1913) reported 1-2 polar and also 3-5 peritrichous flagella, while Woolley (1904, 1905), Calderini (1925), and Sneath et al. (1953 reported one or two polar or subpolar flagella, sometimes two at one pole. Corpe (1954) reported that his strains had polar or peritrichous flagella.

Cruess-Callaghan and Gorman (1935) made a special study of the flagella of their strains. They observed that all strains had a single polar flagellum and in addition some had a few peritrichous (lateral) flagella. They believed that this could be used to distinguish the species, but this has not proved to be so (Leifson, 1956b; Sneath, 1956b).

Conn and his colleagues (Conn and Wolfe, 1938a; Conn, Wolfe and Ford, 1940; Conn and Elrod, 1947) examined several of Cruess-Callaghan and Gorman's strains and found that most strains had a single polar flagellum but that a few had also a lateral flagellum. They also noted that a similar arrangement could occur in strains of Rhizobium, Alcaligenes and other Gram-negative bacteria, and considered that it was a "degenerate peritrichate flagellation"; that is the arrangement was peritrichate, but there were so few flagella that they often appeared to spring from the poles of the organism.

The position was cleared up when it was shown by Leifson (1956a, 1956b) and Sneath (1956c) that strains of Chromobacterium generally possess both a single polar flagellum and also lateral flagella which are distinguishable by their shape, length, curvature and ease of staining. Leifson found th t the polar flagella averaged about 2.2 μ in wavelength and 0.54 μ in amplitude, and the lateral flagella about 1.3 μ in wavelength and 0.45 μ in amplitude, and there were slight differences between different species of the genus (see Nos. 80, 81 and 109 in Appendix II). The polar flagellum often stained very faintly, though he obtained good staining by increasing the concentration of tannic acid in his stain (Leifson, 1951) from 1% to 2%. A few strains possessed only the polar flagellum. These findings are in good accord with my own, and I have found that the two kinds of flagella are also antigenically different. Leifson noted that the number of lateral flagella varied from strain to strain, and was able to obtain variants with a greater number of lateral flagella by subculturing from the edge of colonies spreading in semisolid agar. However, I have found a very marked environmental effect controlling the number of lateral flagella: these appear in large numbers only in cultures grown on solid media (see below). Pijper, Neser and Abraham (1956) report that flagella in Chromobacterium are only found in cultures on agar, which would be explained if they did not detect the single polar flagellum.

It is commonly thought that bacteria are either polar flagellated or peritrichous, and this is used as an important character in many schemes of classification. Leifson and Hugh (1953) and Houwink and van Iterson (1950) report other bacteria which possess both, and there are reports of organisms which would be classified as coliforms if they had not polar flagella (Crawford, 1955; Sreenivasan and Venkataraman, 1956), so this character is of doubtful value in taxonomy. It is interesting to note that Lehmann and Neumann (1899, Vol.1, Pl.23, figs. XI, XII; Vol.2, p.180) observed the two kinds of flagella in Chromobacterium and shrewdly remarked that since some strains had only one type and others had the other, this character would prove of little taxonomic value.

Observations on flagella.

These observations have been published elsewhere (Sneath, 1956c) and the more interesting points are shown in Protocol 2 (Appendix I). All the strains of Chromobacterium were motile. It was noticed that two

forms of flagella were seen on most strains if they were young cultures on agar, and that in old agar cultures or in broth cultures the organisms generally showed only a single polar flagellum. The characteristics of the two forms of flagellum may be summarized as follows:

Polar flagella. These spring from the extreme tip of the organism, are generally 2-4 μ long, and show only one or two complete phases of a wavelength of about 2 μ and an amplitude of about 0.35 μ. more difficult to stain, and stain more faintly, than the lateral flagella.

Lateral (Peritrichous) flagella. These spring from the sides, or may be subpolar, are generally 3-10 μ long, showing many complete phases with a wvvelength of about 1.25 μ and an amplitude of about 0.4 μ. They stain easily and deeply.

These differences are shown diagrammatically in Fig. 1a. Most of the strains show both forms of flagella in young cultures on nutrient agar. However, strains FH, MW, AM, MH, MC and HF showed only the polar flagellum, and lateral flagella have never been observed under any of the conditions examined. With strains NT and PT it was difficult to demonstrate convincingly the polar flagella, but I believe that both strains possessed them. In all the other strains it was clear that the organisms possessed both polar and lateral flagella. Some strains, e.g. BH, only showed occasional organisms with lateral flagella.

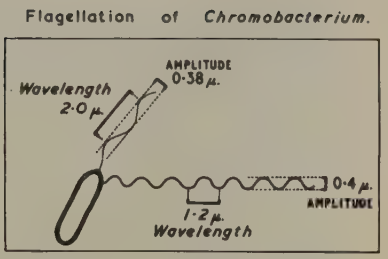
The characteristic flagellation is found both with mesophilic and psychrophilic strains of Chromobacterium and is thus of use in defining the genus. The differences in flagella between the strains do not correlate with any of their other features, so they are not useful in subdividing the genus into species. Strains which lack lateral flagella can occur among either mesophils or psychrophils, and are presumably of the nature of loss-variants. Some photographs of stained films and electron micrographs (kindly prepared by Mr. R. Valentine) are shown in Figs. 2-4 and in Sneath (1956c).

Not all strains show flagella which agree in all details with the descriptions above. For example, in strains AM and MH the single polar flagellum is longer than usual and stains readily, and in a few strains the lateral flagella are rather short, or are of long wavelength. Leifson (1956b) observed a similar variability.

Antigenic difference between polar and lateral flagella.

This has also been reported in detail in Sneath, 1956c. Most of the work was done with strain DK. It was found that immunization of rabbits with formolized suspensions of young organisms grown on nutrient agar and possessing many lateral flagella gave rise to an antiserum which agglutinated such suspensions to a high titre, but agglutinated poorly suspensions which only had polar flagella. On absorption of the serum with organisms which possessed only polar flagella, O and polar H antibodies were removed and a pure antilateral H serum was obtained. The injection of polar suspensions into rabbits gave an antiserum which contained a relatively low titre of this antilateral H component, but evidently there were enough lateral flagella present to give rise to the antibody.

Figure 1a. Diagram of the difference between polar flagella as seen in stained preparations.



Chromobacterium violaceum strain DK

Antigenic difference between polar and lateral flagella

Rabbit antisera x living organisms;– 18hours at 56°C.

IMMUNIZING ANTIGEN	*ABSORBED WITH:-*	Agglutination titres against:-			Content of:-		
		0 cells	POLAR cells	PERITRICHATE cells	ANTI-0	ANTI-POLAR	ANTI-LATERAL
POLAR CELLS	*Unabsorbed*	256	512	512	++	++	++
PERITRICHATE CELLS	*Unabsorbed*	256	256	4,096	++	++	+++
POLAR CELLS	*O cells*	8	128	256	±	++	++
PERITRICHATE CELLS	*Polar cells*	2	2	2,048	-	-	+++

Figure 1b. Serological difference between polar and lateral flagella.

These results are shown below (taken from Sneath, 1956c).

Cross absorption of antisera by suspensions of polar or peritrichate organisms of Chromobacterium sp. strain DK

Serum was absorbed by mixing with an equal volume of 20% (v/v) suspension of packed organisms, and centrifuging after 1 hour at 35°. The titrations were incubated at 56° for 4 hours.

Antiserum to	Absorbed with	Titre against Polar organisms	Titre against Peritrichate organisms
Polar organisms	Unabsorbed	640	1,280
Polar organisms	Polar organisms	20	640
Polar organisms	Peritrichate organisms	40	20
Peritrichate organisms	Unabsorbed	320	10,240
Peritrichate organisms	Polar organisms	40	5,120*
Peritrichate organisms	Peritrichate organisms	40	1,280**

* The titre was not significantly decreased by two further absorptions with polar organisms.

** The titre was decreased to 80 by a second absorption with peritrichate organisms.

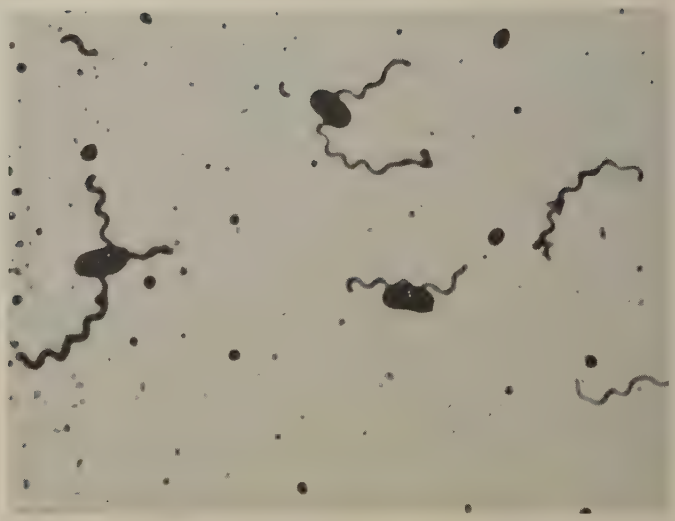


Figure 2. Photomicrograph of a preparation of Chromobacterium strain LW stained for flagella, grown on nutrient agar for 24 hours at 30°. One organism has both polar and lateral flagella. x 2,500.

Figure 3. Chromobacterium strain DK, from 4 hour broth culture. Polar flagella. Electron micrograph, fixed with OsO_4, carbon shadowed. x 13,700.

Figure 4. Chromobacterium strain DK, from 18 hour nutrient agar plate. Lateral flagella (this organism does not show a polar flagellum). Electron micrograph, fixed with OsO_4, carbon shadowed. x 8,400.

It was also shown that polar organisms have (for this lateral H antibody) less than 1/200th of the absorbing power of the same amount of peritrichate organisms. This also suggests that the polar and lateral flagella are antigenically distinct. Since peritrichous suspensions become inagglutinable by this antibody if they are deflagellated by shaking, it is unlikely that the antigen responsible can be an O antigen.

Positive proof of the existence of a polar H antigen was obtained by incubating the agglutination tests for 18 hours at 56°, following a hint by Gardner and Venkatraman (1935) that long incubation at high temperatures was needed to obtain H agglutination of the monotrichate cholera vibrio. With this technique it was possible to show (Sneath, 1956c, Table 3) that there was an antibody fraction in the sera which would agglutinate polar flagellated suspensions but not deflagellated suspensions. Unfortunately it was not possible to prepare a pure antipolar H serum, since the peritrichous suspensions possess also polar flagella (which remove this antibody), and as mentioned above, polar suspensions always give rise to some antilateral H antibody. This is summarized in Fig. 1b.

An antilateral H antibody was similarly obtained with strain BN. Absorption of an anti-BN 'OH' serum with a polar suspension of BN gave a serum which would specifically agglutinate peritrichate suspensions of BN.

In confirmation of the above observations is the appearance of the agglutinates. Lateral H agglutination gives the flocculent masses, which appear within an hour at 56°, typical of H agglutination in classical

peritrichate bacteria like Salmonella. The antilateral H serum at once immobilizes peritrichous cells, or reduces their movement to a jerky and sluggish one. Polar H agglutination gives a fine flaky precipitate, not unlike O agglutination. Antipolar H serum does not immediately immobilize polar flagellated cells, but after a time they collect into poorly motile rosettes and small clumps, unlike the clumps obtained with O antisera, which were, as noted by Arkwright (1927), very motile. Presumably the H antisera act by making the flagella adhesive, and it is only with peritrichous flagella that they immediately become entangled together and their action is thus impeded at once.

The cause of the change of flagella arrangement under different cultural conditions.

Many attempts were made to discover what was responsible for the appearance of lateral flagella in young agar cultures. This has not been satisfactorily explained. There are few lateral flagella (or none) in broth cultures, many in young agar cultures and again few or none in old agar cultures (48 hours or more old). This has been confirmed with strains DK, LG, MK, SH, RV, SL, BH, BN and NT. Strains DK and LG were studied more intensively. Blood agar was the medium which gave the greatest number of lateral flagella. Changes in the following factors did not alter the number of lateral flagella in liquid media to any appreciable extent: anaerobiosis, aeration, growth temperature, age of culture, pH of the medium, concentration of glucose, amino-acids, ammonium, sodium, cyanide, potassium, chloride, phosphate, sulfate, magnesium, or calcium ions. Charcoal had no effect. Addition of granules of agar had little or no effect. Lateral flagella were abundant in young cultures on silica-gel nutrient broth plates and in cultures upon cellophane floating on broth. Presumably the effective viscosity is high within colonies on solid media, and it is possible that this is partly responsible for the appearance of lateral flagella, but addition of gelatin or methylcellulose to broth cultures gave inconclusive results. Almost all this work was with mesophilic strains, and the psychrophils have not yet been investigated.

Spores

Chromobacterium does not appear to form spores. Probably the central spores seen by Fraenkel (1887), Frankland and Frankland (1889), Bréaudat (1906) and Sartory, Meyer and Waeldele (1938a) were artifacts. Corpe (1954) noted occasional spore-like bodies in some strains. None of these reports was supported by tests of the heat-resistance of the supposed spores.

No spores were found in any of my strains. Nutrient broth cultures and nutrient agar cultures grown at 25° for 7 days were heated at 56° for 30 minutes: sterile broth was then added and after incubation at 25° for 4 days it was plated for sterility. None of the cultures survived the heating. In Ziehl-Neelsen stained films occasional oval central acid-fast bodies were seen in strains EC, HB, CA, NT, GA and AM, but they too did not survive heating at 56° for 30 minutes: these were presumably artifacts.

Staining

These organisms stain readily with the usual dyes, and they are not acid-fast. Most authors report them as Gram-negative. Lehmann and Neumann (1899, Vol. 2, p. 262 and table following p. 494) and Calderini (1925) found some strains to be Gram-positive. Grimes (1930) found them Gram-variable. Many workers have noted barred or bipolar staining (well shown by Itzerott and Niemann, 1895 Taf. XV, fig. 89). Neisser (1897) found no metachromatic granules, but Bampton (1913), Calderini (1925) and Zettnow (1899) report polar granules. Burdon (1946) found much "fat" in the cells, which may explain the low specific gravity found by Almquist (1898) and the behaviour with solvents noted by Albertsson (1956). The fatty material is probably poly-β-hydroxybutyric acid (Forsyth, Hayward and Roberts, 1958).

My strains were examined after 18 hours and 4 days growth at 25° on nutrient agar. The methods and the results after 18 hours are summarized in Protocol 1 (see Appendix I), and very similar results were found after 4 days. All strains of Chromobacterium were Gram-negative, though HA, EC, MA and MW tended in young cultures to be Gram-variable. There were often Gram-positive granules at the poles, and Albert's and Neisser's stains for metachromatic granules usually showed deeply staining polar granules. However, these latter usually had the colour of the counterstain, and it was only in strain EC that large and unmistakable blue granules were seen with Neisser's stain. The Gram-positive granules were seen in both young and old cultures, and often resisted decolorization with ethanol for several minutes. They were also seen in a strain of Pseudomonas aeruginosa.

None of the strains was acid-fast. Most showed pronounced barred staining (in the longer forms) or bipolar staining with a central pale area (in the short forms). Sometimes pale oval areas were seen in the longer rods. The barring was of about the same degree after 4 days growth. No capsules were seen, but a mucoid intercellular substance was sometimes present. Corpe (1958) reports that this substance has a fibrillary structure and that it is a polysaccharide. Large globules of fatty material were found after 18 hours in most of the meosphilic organisms, and were generally subpolar in position. The psychrophils seldom contained much "fat." After 4 days growth there was less difference between mesophils and psychrophils, and both groups usually showed a moderate amount of "fat." Stains for cell walls by the method of Webb (1954) and for nuclear material by the method of Murray, Gillen and Heagy (1950) showed no internal cross-walls in the rods, and two or four (or rarely more) nuclear masses in strains DK, LG, HB and NT.

CHAPTER VI

CULTURAL APPEARANCES

The media used as a routine are described in Protocol 3 in Appendix I

Plate cultures

The descriptions of the colonial morphology in the literature are very variable, as would be expected, since the degree of pigmentation and of roughness varies a good deal from strain to strain. Freshly isolated strains generally produce smooth, round, deep violet colonies, which are low convex, semitransparent and glistening. The pigmentation is slight in the first day or two, and then increases, so that particularly with mesophils (Chromobacterium violaceum), the colonies become deep violet or almost black. The pigment may diffuse slightly after several days growth. Many psychrophils (Chromobacterium lividum) give a tough membranous growth.

Nutrient agar plate cultures.

The colonies after 2 days at 20° are generally about 2 mm in diameter, round, smooth, semitransparent, shiny, low convex, and pale violet. Some psychrophils produce rugose colonies with an undulant edge. At 37° or 30° most mesophils produce pigment rapidly, while psychrophils fail to grow at 37° and generally produce very little pigment at 30°. Older colonies may show a metallic sheen. Pigmentation in both groups usually starts in the centre, but may commence at the edge. Some strains produce a tough membranous growth, which is rubbery and has been called "gelatinous" by Corpe (1953) who found that such strains only occur among psychrophils, and that they frequently show variants which are not gelatinous. Corpe reports that yeast extract increases the rubbery quality, and old colonies may become wrinkled. Emulsification is usually easy except in the gelatinous strains.

In my hands both mesophils and psychrophils usually give smooth colonies similar to the description above. The appearance of mesophilic strains grown at 37° is very similar to that seen when they are grown at 25°, except that they grow more quickly at the higher temperature. Some mesophils were culturally and antigenically rough (see Chapter XIV) and these produced flat, raised colonies with a polygonal outline, an undulate or erose edge, and a rough or copperbeaten, dull, granular surface: they gave granular suspensions which autoagglutinated in saline. A few mesophils (strains AM, MW, LW, TV, TA and MH) grew better at 30° than 37°, and if the plates were overdried they sometimes would not grow at all at 37°. The colonies of the psychrophils were more uniform (since none of the strains was very rough) and were smooth, shiny, round, convex with an entire edge. Occasionally they became slightly umbonate. There was little rubbery consistency before 3 days growth at 25°.

Both mesophils and psychrophils gave colonies with a diameter of about 0.5 mm after 24 hours at 25°, and about 4 mm in diameter after 5 days at 25°. Small inocula often failed to grow (see Chapter VIII). Pigment was visible as a rule after 2 days at this temperature, but psychrophils produce pigment more slowly than mesophils, and may show concentric rings of pigmentation. In both groups pigment was generally first seen in the centre of the colonies, but occasionally the edge showed it first. Almost all strains threw off pale or colourless variants, sometimes showing a pale yellow nondiffusing pigment, and all the gelatinous strains yielded occasional nongelatinous colonies. After 7 days many psychrophils were gelatinous (tough and rubbery), and the colonies could be detached whole with a needle. The colonies had a finely granular centre but were otherwise amorphous. The degree of pigmentation and of gelatinous growth is shown in Table 3.

Nutrient gelatin plates.

The colonial morphology is even more variable than on nutrient agar, since it is also affected by the degrees of liquefaction of the gelatin. Most workers report that colonies of mesophils rapidly sink into round cups of liquefied gelatin, while psychrophils, which liquefy gelatin slowly if at all, give flat colonies with an irregular or lobate edge. The strain described by Ajtai (1887) as Bacillus violaceus diffusus (No. 111) seems to have been a psychrophil which produced an effuse or swarming edge. Violet pigment and membranous growth are well developed on nutrient gelatin. My own observations are in keeping with the descriptions above: surface streaked plates incubated at 20° showed round colonies of mesophils which sank into the medium, and flat lobate colonies of psychrophils. Only a few psychrophils (strains EC, DA and IN) showed any liquefaction of the medium after 5 days, and it was very slight. Pigmentation was well-marked.

Deep colonies.

These are not very characteristic. Pigmentation is slight, and the colonies are white and lenticular. In gelatin the mesophils show a spherical zone of liquefaction after a few days.

Stroke cultures

The growth on nutrient agar stroke culture is usually reported as smooth, shiny and deep violet after a few days. The edges are reported as even, undulate or ciliate. Gelatinous psychrophils produce a tough membrane. At 25° most of my strains gave a shiny flat growth with a lobate edge and an undulating surface. Psychrophils differ little in appearance from mesophils. Old cultures are deep violet as a rule and may show a coppery reflex.

Gelatin stab cultures

The mesophils and psychrophils differ considerably in gelatin stabs. The former grow in the depth along the stab as a filiform white streak, and rapidly produce liquefaction. This is generally infundibuliform, but

Table 3. Degree of violet pigment and of gelatinous growth in cultures on nutrient agar grown for 7 days at 25°.

Strain	Violet pigment	Gelatinous growth	Strain	Violet pigment	Gelatinous growth
	MESOPHILS			PSYCHROPHILS	
FH	++	-	NT	+	+++
MK	+++	-	EA	+	+++
BH	+++	-	EB	+	+
BN	+++	-	EC	+	+
AM	++	-	GA	+	-
MW	++	-	DA	++	-
TV	+	-	NC	++	+
LG	+++	-	MA	++	+
SL	+++	-	MB	+	++
RV	++	-	MC	++	+
DK	+++	-	HA	++	++
TA	++	-	HB	++	++
TB	++	-	HC	+++	+++
PT	+++	-	HD	++	+
LW	++	-	HE	+++	++
SH	++	-	HF	+	++
RT	+++	-	IN	+	+++
MH	++	-	CA	+++	+++
BM	+++	-	PB	++	-
			RU	++	++
			ED	+	-
			EF	+	-
			BC	++	+
			OTHER BACTERIA		
Chromobacterium viscosum			GR	-	(viscid)
Chromobacterium iodinum			TE	(crystals)	-
Chromobacterium ianthinum			TI	-	(viscid)

Symbols: Pigment, + to +++ = increasing degrees of violet pigmentation from pale violet to violet-black.

Gelatinous growth, + to +++ = increasing degrees of jelly-like consistency, from slight to tough and membranous.

may be napiform; it is well-marked after 5 days at 20°, and after 2 or 3 weeks the whole tube may become fluid. There is constantly a violet pellicle and a grey-violet deposit. The psychrophils grow very poorly below the surface and give a raised violet button of surface growth. Liquefaction is absent or very slow. Of my psychrophilic strains only EA, DA and IN showed any liquefaction after 14 days at 20°, and it was very slight. These results are shown in detail in Protocol 3, Appendix I.

These findings are similar to those reported in the literature. Leifson (1956b) has confirmed the difference between psychrophils and mesophils in the rate of liquefaction. Kufferath (1915) noted that rapidly liquefying strains would only slowly liquefy 70% gelatin.

Growth in fluid media

The appearance in broth or other fluid media depends largely upon the degree of roughness, the ability to grow anaerobically, the degree of pigmentation and the membranous consistency of the growth. The descriptions in the literature vary very widely, as expected. In my hands, nutrient broth cultures grown at 25° for three to five days generally show a violet ring adherent to the glass at the surface of the medium. This ring is powdery in nongelatinous strains, and in gelatinous strains it is thick, viscous and shows hanging fronds of growth even in psychrophils with a minimal degree of gelatinous growth on agar. Turbitidy is less with mesophils than psychrophils, and is less with rough strains and gelatinous strains. There is usually some deposit which has the consistency of the violet ring. Mesophils generally show a fragile pellicle which easily sinks; psychrophils, if gelatinous, may sometimes show a tough thick pellicle, but frequently do not. Old cultures become very alkaline (Wolff, 1911): I have found the pH to be generally about 10.0 after two weeks at 25°.

Growth on special media

Blood agar plates.

The colonial form and the degree of pigmentation are much the same as on plain nutrient agar. Several authors (Dodd, 1941, p.21; Sippel et al., 1954; Lesslar, 1927; Audebaud et al., 1954; Floch and de Lajudie, 1943; Sneath et al., 1953; Black and Shahan, 1938) have noted haemolysis around the colonies which seems to have been a β haemolysis, though Sippel (1955, p.39) describes an outer greenish zone and regarded it as a mixed α and β type of haemolysis. These strains were all mesophils. In studying psychrophils Bampton (1913) and Deshusses and Noval (1939) found no haemolysis. This distinction between the two forms seems to be fairly reliable, though the old work often does not mention which animal's red cells were used. In my experience, growth on 5% horse blood agar is a little better than on nutrient agar but is otherwise the same: small inocula grow readily (see Chapter VIII) and as a rule mesophils produce a partial β haemolysis with a diffuse edge, while psychrophils show no haemolysis.

Potato slopes.

The behaviour on potato is fairly constant. Most authors have observed good growth and good pigmentation on this medium, but a few disagree. Frankland and Frankland (1889), Dyar (1895), Bampton (1913) and Cruess-Callaghan and Gorman (1935) observed strains which grew poorly, and Macé (1888c), Eisenberg (1891, p. 421), Germano (1892), Lehmann and Neumann (1899, Vol. 2, p. 263), Mehta (1925) and Calderini (1925) observed strains which produced little violet pigment, but instead gave brownish, yellowish or greenish growths. It seems that psychrophils often produce little violet pigment on potato, but that mesophils usually produce much. The growth is moist and shiny, sometimes spreading. The violet pigment does not diffuse greatly into the medium, but the potato may become stained brownish or greenish. Growth is much the same on glycerinated potato as on plain potato.

On potato slopes at 25° all my strains grew well, giving smooth undulant shiny growth with a diffuse edge, sometimes spreading across the medium. One strain (RV) gave a wrinkled growth. Violet pigment was heavy with all the mesophils after 7 days. Psychrophils, though growing well, showed much less violet pigment. After 2 weeks, only strains NT, MA and HE produced deep violet pigment, strains EB, EC, GA, MB, MC and HF gave only a yellowish growth, and the remaining eleven strains gave slight or moderate degrees of brown colour tinged with violet. The membranous property was poorly developed on potato.

Cruess-Callaghan and Gorman (1935) used the degree of growth on potato to differentiate the species, but it has not been useful in my hands since all strains grew well and the degree of growth is not easy to estimate when the pigmentation varies so widely.

Löffler's inspissated serum slopes.

Strains which were probably mesophils are reported to liquefy this medium (e.g. Eisenberg, 1886, Tab. 2; Fuller and Johnson, 1899; Dodd, 1941, p. 25; Lesslar, 1927; Minett, 1913). Deshusses and Novel (1939) and Bampton (1913) reported slight or doubtful liquefaction with psychrophilic strains. All my strains grew well at 25°, giving soft, shiny growth, but pigmentation was erratic. All the mesophils produced moderate digestion after a week, so that the growth sank into a furrow in the medium, and after two weeks the medium collapsed. Psychrophils gave little or no digestion in this time (see Protocol 13 in Appendix I). The mesophils are not as proteolytic as Serratia or Pseudomonas aeruginosa.

Other media.

Strains have been reported to grow on Bordet-Gengou agar (Dodd, 1941, p. 21), on Sabouraud's glucose agar (Deshusses and Novel, 1939; Waeldele, 1938, p. 73; and personal observations), on tellurite media, which are blackened (Gosio, 1905; Gloger, 1906), on Jerusalem artichoke (on which a green growth is produced, Thiry, 1900, p. 112; Calderini, 1925), and on MacConkey agar (Cunningham and Raghavachari, 1924; the colonies are whitish and later violet). Bampton (1913) found they produced no fluorescence on Neutral Red agar. Glycerol agar and glycerol potato allows good growth and pigmentation (Dodd, 1941, p. 21; Waeldele, 1938, p. 75). Some strains liquefy coagulated white of egg (Audebaud et

al., 1954; Waeldele, 1938, p. 78), others do not (Deshusses and Novel, 1939).

Smell

Gauducheau (1907) reported that a mesophilic strain smelt of bitter almonds, and mesophils do in fact smell quite strongly of hydrogen cyanide and also of ammonia (see page 311). Macé (1887a) reports a psychrophilic strain as smelling of cream cheese or butyric acid, and I find that psychrophils have a penetrating odour somewhat reminiscent of the latter. Waeldele (1938, p. 69) reports a smell of strawberries.

CHAPTER VII

RESISTANCE

Resistance to heat

Strains of Chromobacterium have the usual resistance to heat found in most vegetative bacteria. Most authors agree that cultures are sterilized by heating for 30 minutes at 56° or over, and by 100° within a minute (Bampton, 1913; Woolley, 1904, 1905). However, Deshusses and Novel (1939) and Ward (1898) report survival after 30 minutes at 55°, but this may have been due to faulty technique. Godfrin (1934, p. 86) reports that strains are rapidly killed at 35°, which is presumably an error. They may survive 50° for 30 minutes (Sippel, 1955, p. 39).

Broth cultures of all my strains were sterilized by heating at 56° for 30 minutes. Bampton (1913) reported that Bacillus violaceus was less resistant to heat than Bacillus membranaceus amethystinus. Although his technique was followed as far as possible this test was not found reliable (see Protocol 4 in Appendix I) and the most sensitive strains were equally distributed between mesophils and psychrophils, and had no other distinctive properties in common.

Resistance to phenol

The testing method used was as follows: 0.8 ml of a nutrient broth culture grown for 2 days at 25° was mixed with 0.2 ml of 5% (w/v) aqueous phenol, and incubated in a water bath at 20°C. After 5 and 10 minutes, 0.05 ml samples were transferred to 5 ml of nutrient broth from which a loopful was plated on blood agar and also incubated for sterility. The results were very uniform. A few strains showed surviving organisms after 5 min., but the 0.05 ml samples were always sterile after 10 min. The final concentration of phenol (1 in 10,000) was not inhibitory in control experiments (as was reported by Wittlin, 1896). Galeotti (1892) reported that 1% of phenol did not inhibit growth, which seems unbelievable. Some strains of Chromobacterium are more sensitive to phenol than are "Chromobacterium viscosum" (strain GR) or "Chromobacterium iodinum" (strain TE), which showed many surviving organisms after 10 minutes.

Resistance to antibiotics

Almost all the records are on virulent mesophilic strains, to which the following remarks apply. All strains are highly resistant to penicillin (100 units/ml) and are sensitive to streptomycin, chloramphenicol, chlortetracyclin (aureomycin) and oxytetracyclin (terramycin) at levels which are readily attainable in the blood stream (Sneath et al., 1953; Hans and Bicknell, 1953; Audebaud et al., 1954; Sippel et al., 1954 ; Darrasse et al., 1955; Joubert and Nguyen-Van-Liem, 1957, and see Protocol 5 in Appendix I). They are moderately sensitive to dihydrostreptomycin (Hans and Bicknell, 1953; Sippel, 1955, p.49), and sensitive to tetracyclin and neomycin (Darrasse et al., 1955). They are slightly sensitive to erythromycin and framycetin (soframycin) (Darrasse et al., 1955; Joubert and Nguyen-Van-Liem, 1957), and to polymyxin B (Sippel, 1955, p.49). They are resistant to bacitracin (Joubert and Nguyen-Van-Liem, 1957; Darrasse et al., 1955; Sippel, 1955, p.49), and to sulfadiazine (Sneath et al., 1953). The inhibitory concentrations with streptomycin, aureomycin and terramycin were 4 μg/ml, 2 μg/ml and 39 μg/ml respectively (Sippel et al., 1954). Strains resistant to streptomycin can arise in vitro (see Protocol 5).

Coffey, Hillegas, Knudsen, Koepsell, Oyaas and Ehrlich (1954) found Chromobacterium to be resistant to azaserine. Cercos (1951) and Cercos and Castronovo (1951) found it sensitive to fungocin and to the alkaloid vinalin.

The resistance to penicillin seems to be due to penicillinase. Czekalowski (1950) observed that a strain of Chromobacterium inactivated penicillin, and Dr. M.R. Pollock kindly tested strains MK and HB for the enzyme. Both strains produced a low level of penicillinase after induction with penicillin.

Longevity

Strains of Chromobacterium soon die out in culture. In my experience nutrient agar slopes die out in two weeks at 20° as a rule. Ramchandani (1930) had similar experiences. In broth they live longer, usually one month or more ar 20°. Mesophils in broth survive for some weeks at 37°, but die in a week or two at 4°. Psychrophils live for some weeks at 4° but soon die at 30° or over. Stab cultures in nutrient agar live longer than broth cultures and are best for transport by post. It is possible that the alkalinity of old cultures is responsible for death, and the semianaerobic conditions of stabs delays this alkalinity. Eltinge (1956) finds that agar slopes covered with sterile mineral oil (liquid paraffin) will survive for 1 year at 20°. Darrasse et al. (1955) found that a culture in blood broth of a mesophil survived for a year at 30°. I have kept a few strains at -78° in a solid carbon-dioxide cabinet as suspensions in broth, and all were alive after 1 year. In recovering organisms from old cultures it is advantageous to add yeast extract to the medium, since this gives a higher percentage of viable cultures. Broadhurst (1921) had noted the beneficial effect of "vitamins" in bean extracts on the recovery from old cultures.

As noted by Rhodes (1950) these organisms do not live for long when

Figure 5. Photograph of a nutrient agar plate culture of Chromobacterium strain LG on which has been printed by sunlight a picture of Paul Ehrlich. x2.

freeze-dried. My experience has been that when suspended in horse-serum containing 5% of glucose and lyophilized and sealed in vacuo, the cultures usually survive for about a year.

Resistance to other agencies

Ward (1897; 1898) noted that psychrophilic strains were very sensitive to sunlight, and found blue light to be most lethal. He was able to "print" photographic negatives onto plate cultures (see Fig. 5). Burge and Neill (1915) and Berger, Haas, Wyss and Stone (1953) found Chromobacterium to be one of the bacteria most sensitive to ultraviolet light, which may act through peroxide production (see Chapter VIII). Baugh and Clark (1959) studied the photodynamic effect of erythrosin on Chromobacterium. Weinberg (1953) found it moderately sensitive to triphenyltetrazolium chloride. Lichstein and Soule (1944) found it was inhibited by 0.03% of sodium azide. Gray (1954) found it resistant to benzenehexachloride. Schmidt (1891) found that like other bacteria it was not killed by vigorous shaking. Kohn (1906) found that it would not grow in 10% glucose in 1% ammonium phosphate. Roncali (1892) noted that cultures of tubercle bacilli would inhibit it.

CHAPTER VIII

THE CATALASE EFFECT

It is well known that the plague bacillus (Pasteurella pestis) is unable to grow aerobically upon nutrient agar from small inocula. Herbert (1949) showed that this is due to inhibition by traces of peroxide in the medium and that the inhibition is overcome by minute amounts of catalase. The only other well-known examples of this phenomenon are shown by Pasteurella septica (Jordan, 1952) and Shigella dysenteriae (Proom, Woiwod, Barnes and Orbell, 1950). This phenomenon has been observed with strains of Chromobacterium (Sneath, 1955), and the inhibition was abolished by blood, manganese dioxide, haematin, Fildes' extract and by anaerobiosis as well as by small amounts of catalase. Twelve strains of mesophils all showed the effect.

However, the phenomenon is probably not uncommon, and recently it has been reported in Haemophilus pertussis (Mazloum and Rowley, 1955) and in various species of Bacillus (Taylor, 1956, who also confirmed the phenomenon in Chromobacterium).

The origin and nature of the inhibitory substance is still rather obscure. There seems general agreement that it is a peroxide, since catalase, haematin, manganese dioxide and other substances which decompose hydrogen peroxide are able to destroy it, but it is possible that it may be an organic peroxide. Taylor found that it did not diffuse through cellophane, though my own experiments suggest that it does. Proom et al. (1950) found that it was formed when broth and agar were autoclaved together, but not if they were autoclaved separately and then mixed at 56°. Barry, Conalty, Denneny and Winder (1956) showed that peroxide was formed when citrate or reducing sugars were heated in the presence of traces of manganese, and suggest that this may be the mechanism of formation.

It is clear that the phenomenon does not depend simply on the absence of catalase in the bacteria. Both Chromobacterium and Pasteurella pestis produce catalase, while some streptococci which contain no catalase can produce enough peroxide to inhibit other bacteria (Thompson and Johnson, 1951). It seems that it depends rather upon the great sensitivity to peroxide of those bacteria which show the phenomenon. Peroxide is well known to be produced by ultraviolet light, and this would also explain the great sensitivity of Chromobacterium to ultraviolet light and to sunlight (Berger et al., 1953; Ward, 1897).

The technique used by me was a simple one. A ditch was cut out of a nutrient agar plate and the agar removed and melted at 100°, and cooled to 50°. The substance to be tested was added and the agar poured back into the ditch. After cooling and drying a crop of a suitable culture was run across the plate and ditch, and when dry the plate was incubated at 25°. With undiluted cultures growth usually occurs along the whole streak. With dilutions containing about 10^4 to 10^6 organisms per ml (i.e. cultures diluted 100 to 10,000 times) there are no colonies on the streak except near the ditch (if a peroxide-destroying substance was added). The strains reported on by Sneath (1955) were all mesophils (strains FH,

MK, BH, BN, LG, SL, RV, DK, and AM). Since then six psychrophils (strains NT, EB, HB, MA, IN, RU) have been examined and they also show the effect. With all these strains 0.1 μg/ml of pure horse liver catalase, or 10 μg/ml of haematin, was sufficient to abolish the inhibition. Some strains are more easily inhibited than others, and the degree of inhibition varies with the batch of agar. Photographs of some plates are shown in Figs. 6-9.

CHAPTER IX

METABOLISM AND NUTRITION

Growth curves

Jennison (1932; 1935) studied a mesophilic chromobacterium. The usual sigmoid growth curve was found, consisting of a lag period of two hours followed by six generations of logarithmic growth, and a stationary phase in which the viable count was about 2×10^8 organisms/ml. In aerobic but nonaerated broth cultures the mean generation time in the logarithmic phase was 55 minutes at 22°, 34 minutes at 27°, 23 minutes at 32°, and 25 minutes at 37°. In my experience the viable count of aerobic but nonaerated nutrient broth cultures is usually about 5×10^8 organisms/ml after 24 hours at 25°. Viable counts on the surface of nutrient agar are unreliable, because of the catalase effect (see last Chapter) but are reproducible on blood agar or on agar containing 5% of the peptic blood digest of Fildes (1920).

Gaseous metabolism

All strains of Chromobacterium will grow aerobically, but the mesophilic strains are also facultatively anaerobic. Several early authors report strictly aerobic strains which were psychrophils (e.g. Germano, 1892; Fuller and Johnson, 1899). Some mesophils are recorded as strict aerobes (Minett, 1913; Audebaud et al., 1954) but this may be due to use of the pyrogallol sodium hydroxide technique, since strains need a trace of carbon-dioxide for growth (Galeotti, 1892; Valley and Rettger, 1925a; 1925b; 1927). They do not need an increased CO_2 tension, however, and are not inhibited by 25% CO_2 in air (Dodd, 1941, pp. 22, 67). Kladakis (1890) found that coal gas was inhibitory.

In testing the strains, nutrient agar plates were inoculated with a loopful of a young broth culture and incubated for 4 days at 25° in an atmosphere of hydrogen with a palladium catalyst. All the mesophils (i.e. Chromobacterium violaceum) grew moderately well without producing pigment. The four strains which did not ferment glucose anaerobically (LW, SH, RT and MH) grew a little less well than the others. Of the psychrophils (i.e. Chromobacterium lividum), a few gave scarcely visible growth, and the remainder did not grow. These results are shown in Table 4.

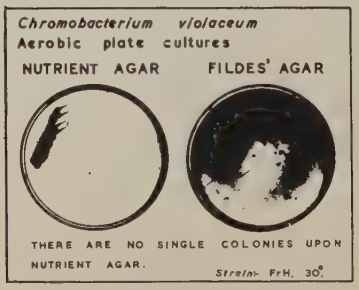


Figure 6. The catalase effect: inhibition of single cells on aerobic nutrient agar. Chromobacterium strain FH.

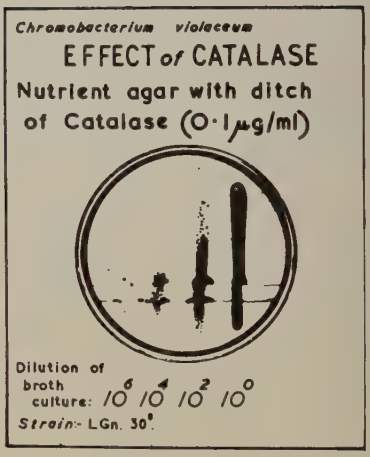


Figure 7. The catalase effect: prevention of inhibition by catalase. Chromobacterium strain LG.

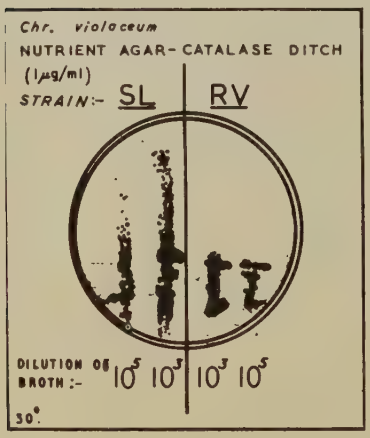


Figure 8. The catalase effect: Effect of catalase, Chromobacterium strains SL and RV.

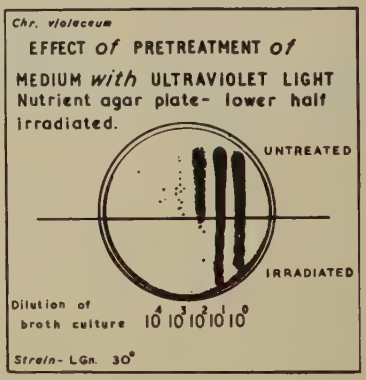


Figure 9. The catalase effect: Increase of the inhibition by pretreatment of medium with ultraviolet light. Chromobacterium strain LG.

Growth temperature

The temperature range for growth is characteristic of the two species of Chromobacterium, as was first realized by Cruess-Callaghan and Gorman (1935) and this is one of the most useful features for distinguishing them in early descriptions. Apparently Havemann (1894) first reported growth of Chromobacterium at temperatures close to 0°. The upper and lower limits for growth of mesophils (C. violaceum) are 37 to 44° and 8 to 16°. The upper and lower limits for psychrophils (C. lividum) are 25-35° and 0-4°. This is shown in Table 4 and Protocol 6 in Appendix I. It can be seen from the protocol that the optimum temperature is closer to the upper limit than to the lower (as seems to be generally true of bacteria according to Ingraham, 1958) being usually 30-37° for mesophils and 25-30° for psychrophils. In particular, all the mesophils grew within 7 days on moist nutrient agar slopes (not necessarily on dry ones) at 37°, and none grew in 7 days at 2°. Conversely, none of the psychrophils grew in this time at 37° and all grew at 2° (except strain EA, which grew at 4°). The mesophils usually produced abundant pigment at any temperature at which they would grow, but psychrophils produced little pigment at 30°, and it was most abundant at temperatures below the optimum for growth.

Two strains sent by Dr. Eltinge belonging to the variety of the psychrophils which she calls "var. mesophilum" (see p. 252) were tested: these were strains ED and EF in Table 2 (p. 262). In my hands they would not grow above 35°, and they do not seem to form a clear-cut variety, though they would not grow at 2°. They are best regarded as aberrant psychrophils.

Ingraham (1958) in an illuminating study has shown that most so-called psychrophils are simply bacteria which grow relatively rapidly at 0° to 4°. Their optimum is usually well above 20°. This seemd true also of the psychrophilic chromobacteria. True psychrophils with an optimum below 20° seem to be very rare.

pH for growth

Chromobacteria grow over a wide range of pH. From the work of Schülter (1892), Deeleman (1897) and Audebaud et al. (1954) it seems that this range is about pH 5 to pH 9. Valley and Rettger (1927) report growth at pH 4.4. The strains studied here were examined by streaking loopfuls of young broth cultures onto nutrient agar plates of pH 4.0 to 10.0 in steps of 1.0, (checked with suitable indicators), and incubating at 25° for 4 days. The behaviour of both mesophils and psychrophils was very uniform: all strains grew at pH 6.0 and pH 9.0, and none grew at pH 4, pH 5, or pH 10. The optimum seemed to be between pH 7 and pH 8.

Tolerance of salt

This was tested by streaking loopfuls of young broth cultures onto nutrient agar plates containing added sodium chloride, and incubating

Table 4. Metabolism and nutrition. (All strains grew aerobically, all grew at pH 6 and pH 9, and all except GR grew in Koser's citrate.)

Strain	Anaerobic growth (4 days at 25°)	Growth in 7 days at 25° on agar containing: 3.5% NaCl	6.5% NaCl	Alkalinity on Simmon's citrate agar at 25° (2 days)	(7 days)	Highest growth temperature	Lowest growth temperature
			MESOPHILS				
FH	++	+	-	B	B	44°	10°
MK	++	++	-	b	B	37°	10°
BH	++	+	-	G	b	44°	16°
BN	++	tr	-	b	B	44°	16°
AM	++	++	-	B	B	37°	10°
MW	++	+	-	b	B	37°	10°
TV	++	+	-	B	B	37°	10°
LG	++	++	-	G	B	44°	10°
SL	++	++	-	b	B	37°	10°
RV	++	++	-	b	B	37°	10°
DK	++	++	-	b	B	37°	16°
TA	++	++	-	B	B	37°	10°
TB	++	++	-	B	B	37°	10°
PT	++	++	-	b	B	37°	10°
LW	+	++	-	b	B	37°	10°
SH	+	++	-	b	B	37°	16°
RT	+	++	-	G	B	37°	16°
MH	+	++	-	b	B	37°	10°
BM	++	NT	NT	NT	NT	37°	10°
			PSYCHROPHILS				
NT	-	+	-	B	B	25°	2°
EA	-	tr	-	B	B	30°	4°
EB	-	+	-	B	B	30°	2°

EC	-	+	-	B	B	30°	2°
GA	tr	++	-	B	B	35°	2°
DA	-	++	-	B	B	30°	2°
NC	-	++	-	B	B	30°	2°
MA	-	tr	-	B	B	30°	2°
MB	-	++	-	B	B	30°	2°
MC	tr	++	-	B	B	30°	2°
HA	-	tr	-	B	B	30°	2°
HB	-	++	-	B	B	30°	2°
HC	-	++	-	B	B	25°	2°
HD	-	++	-	B	B	30°	2°
HE	-	++	-	B	B	30°	2°
HF	tr	tr	-	B	B	30°	2°
IN	tr	tr	-	B	B	35°	2°
CA	-	tr	-	B	B	30°	2°
PB	tr	++	-	B	B	30°	2°
RU	-	tr	-	B	B	30°	2°
ED	-	NT	NT	NT	NT	35°	10°
EF	-	NT	NT	NT	NT	35°	10°
BC	-	NT	NT	NT	NT	30°	2°
			OTHER BACTERIA				
GR	tr	+++	+	G	G	37°	10°
TE	-	+++	+++	G	b	37°	10°
TI	-	++	-	B	B	37°	10°

Symbols: - = no visible growth, tr = growth visible with a lens, + = scanty growth visible without a lens, ++ = good growth, +++ = profuse growth, G = green, no change (c. pH 6.9), b = blue green, slightly alkaline (c. pH 7.2), B = blue, strongly alkaline (c. pH 7.5), NT = not tested, GR = "Chromobacterium viscosum", TE = "Chromobacterium iodinum", TI = "Chromobacterium ianthinum" Gilman.

for 7 days at 25°. All the chromobacteria grew on agar containing a total of 3.5% of NaCl, and none grew on 6.5% salt agar (Table 4).

Attack on carbohydrates

Gas production. All authors agree that Chromobacterium does not produce gas from carbohydrates in the usual fermentation tubes, except for Audebaud et al. (1954) who report gas from sucrose and trehalose only!

Carbohydrate catabolism. As shown in Chapter XI most mesophils produce acid from glucose anaerobically (i. e. fermentatively) while some mesophils and all psychrophils only do so aerobically. Dr. A.T. James and Miss Joan Webb kindly examined the products of glucose catabolism (as described in Sneath, 1956b) with three strains. Strain MK, a typical mesophil, produced acetic and n-butyric acids and probably some succinic and lactic acids, and a large amount of an unidentified nonvolatile acid. There was also a trace of formic acid. Strain LW, a nonfermenting mesophil produced mainly acetic and succinic acids. Strain HB, a psychrophil, produced a little acetic, succinic and lactic acids and several unidentified nonvolatile acids. There was probably some gluconic acid in all three cultures. It is known that the pathways of glucose catabolism differ greatly in bacteria. In Pseudomonas the main pathways are aerobic, through gluconic acid and pentoses, while in Serratia and Escherichia anaerobic pathways predominate yielding acetic, lactic, formic and other acids and often 2:3 butanediol, acetylmethylcarbinol and hydrogen (Lewis, Blumenthal, Weinrach and Weinhouse, 1955; Lemoigne, 1919; Pederson and Breed, 1928; Neish, Blackwood, Robertson and Ledingham, 1948). The fermentation of MK resembles the mixed acid fermentation of some coliform bacteria, but the other two are not easy to interpret.

Nutritional requirements

Strains of Chromobacterium have simple nutritional requirements. They will grow on media consisting of inorganic salts plus asparagine or plus ammonia and a variety of sources of carbon such as glucose, lactate, succinate, and citrate (Sullivan, 1905; Mehta, 1925; Kohn, 1906). Most authors have found tham to grow in Koser's citrate ammonium phosphate medium (Gilman, 1953; Audebaud et al., 1954; Corpe, 1954). Leifson (1956b), however, using Simmon's citrate agar found that though all psychrophilic strains and many mesophilic strains grew rapidly, some mesophils grew slowly or not at all. He used this to subdivide the mesophils into subgroups (see Chapter I).

Waeldele (1938, pp. 83-86) and Sartory, Meyer and Waeldele (1938a) suggest that polyhydric alcohols are not utilized for growth. Quastel, Scholefield and Stevenson (1952) found pyruvic acid oxime was not utilized as sole nitrogen source, and de Ley and Vandamme (1955) found that 2-keto-D-gluconic acid was not utilized as a carbon source. Linardos and Cleverdon (1955) state that all strains require organic nitrogen (whatever that may mean) and found that pyruvate gave better growth than glucose: most strains grew well on an ammonium and salts medium with

0.25% pyruvate and 20 μg each of threonine, cystein, and glutamic acid per ml. DeMoss and Happel (1955) reported methionine to be essential for growth in their strain. Later, DeMoss and Happel (1959) working with strain RT (ATCC 553) reported that it required either D- or L-methionine, or methionine sulfoxide, or else trace amounts of vitamin B_{12} (cobalmin), but these were only required for rapid growth. They suggest that the organism can synthesize vitamin B_{12} but only at a slow rate, and since the vitamin is needed for methionine synthesis, this limits the growth rate in media lacking this amino-acid. They suggested that the organism could be used to assay vitamin B_{12}. Psychrophils and other mesophilic strains have not been examined for methionine or cobalmin requirements. Dmitrevskaia (1936) attributes the frequent loss of pigmentation on subculture to a vaguely described state of "salt starvation."

The nutritional requirements of my strains were studied in Koser's citrate medium (Koser, 1923) made with precautions to avoid contamination with growth factors. Tubes of medium were inoculated with a straight wire dipped in young broth cultures, and incubated at 25° for 7 days. When growth was seen the tube was subcultured with a straight wire into another tube of medium and similarly incubated. All strains grew slowly and grew also in the subculture. This suggests that there is no absolute requirement for any growth factors. This was confirmed by Sneath (1956b) for eight mesophils and two psychrophils, which could be serially subcultured in the ammonium medium of Mandelstam (1954) containing lactate and no glucose.

However, there were considerable differences in the rate at which citrate was utilized. This was tested in Simmons' citrate agar slants (Simmons, 1926). It is possible that Koser's and Simmons' media are testing for different things. The former should be free from growth factors and should therefore test both for citrate utilization and for independence of growth factors. The latter may do this, but if there are growth factors in the agar it may be testing only for the ability to utilize citrate for growth. Growth factors were therefore deliberately added to Simmons' medium in order to test only for citrate utilization: 0.01% of Difco yeast extract was added which was too little to allow growth by itself. The slants were streaked with small loopfuls of young broth cultures and incubated at 25°. All psychrophils grew and made the medium alkaline within 2 days, and some mesophils did likewise. A few mesophils grew slowly and took a week to make it alkaline (see Table 4), but they did not have any other property in common and do not seem to form a very distinct variety.

CHAPTER X

PIGMENTATION: THE PROPERTIES OF VIOLACEIN AND SOME OTHER BACTERIAL PIGMENTS

Violacein

The characteristic violet pigment produced by Chromobacterium is insoluble in water and diffuses through media only very slightly. It has been called janthin (Lehmann and Neumann, 1899, Vol. 2, p. 55, not the ianthin of Moseley, 1877). The first careful study was made by Schneider (1894) and Seppilli (1931) has briefly reviewed the literature.

Solubility. Violacein is insoluble in water, chloroform, carbon disulfide, benzene (Schneider, 1894), toluene (Bampton, 1913), light petroleum (Reilly and Pyne, 1927), and in diethyl ether free from ethanol (Strong, 1944; personal observations). It is soluble in ethanol (to the extent of 0.1% according to Reilly and Pyne), acetone, amyl alcohol (Calderini, 1925), methanol and glycerol (Friedham, 1932a). In aniline it gives a green solution (Waeldele, 1938, p. 103). It is slightly soluble in glacial acetic acid giving a blue solution (Bampton, 1913), and in crude ether, giving a more reddish solution than in alcohol (Bergonzini, 1880; personal observations). It is most soluble in pyridine (to the extent of 0.5% according to Wrede and Rothhaas, 1934a) and in dimethylformamide (personal observations).

Reactions. Schneider (1894) listed many of the reactions of violacein which have been confirmed by others, using solutions in ethanol. With weak acids (e.g. acetic) it becomes blue, with mineral acids it becomes blue-green (e.g. hydrochloric acid) or green (e. g. emerald green on adding 10% of concentrated sulfuric acid). The colour is stable and returns to violet on neutralization. Nitric acid turns it yellow. With sodium or potassium hydroxide the solution becomes green but in a few minutes turns brownish red and after this the violet colour is not restored by neutralization. Ammonium hydroxide turns it blue-green, and a blue precipitate may form.

Chlorine water, bromine water, chromates and permanganates bleach the solutions at once (Wrede and Rothhaas, 1935a). Hydrogen peroxide does not unless heated (Lasseur and Girardet, 1926). Nitrous acid yields a brown precipitate and removes one atom of nitrogen (Reilly and Pyne, 1927). On reduction with zinc dust in acetic acid it is bleached, and Wrede and Rothhaas found the colour to return on aeration. It is also reduced to colourless compounds by thiosulfate, ammonium sulfide, sodium sulfide, or palladium and hydrogen (Audebaud et al., 1954; Waeldele, 1938, p. 95; Friedham, 1932a) and the violet is restored by cautious oxidation with chromate or hydrogen peroxide. It gives a green colour with phenolphthalein (Audebaud et al., 1954).

It is bleached by strong light (von Eisler and von Portheim, 1914; Friedham, 1932a) and is not fluorescent in ultraviolet light (Sippel, 1955, p. 41). It dyes silk and cotton without a mordant but the colour fades in the light (Lasseur and Girardet, 1926).

On treating ethanolic solutions of pigment from strains MK, RV, NT

and HB with equal volumes of the following reagents, the corresponding colour changes were seen: 10% KOH: green, turning reddish and then brown in a few minutes; 50% HNO_2: yellowish orange; 50% H_2SO_4: emerald green, stable for some hours; glacial acetic acid: blue, stable; 2M-HCl: blue-green, turbid, stable; H_2O_2 (10 vol.%): remains violet, stable at 20°, but decolorized at 80°; 0.1 M-$FeCl_3$: pale yellow. On reduction with 25% acetic acid and zinc dust it was decolorized. With 0.5% $NaNO_2$ in 10% acetic acid it became pale brown and turbid. It did not give a red colour with the indole reagent (p-dimethylaminobenzaldehyde in dilute hydrochloric acid).

Chromatography. Audebaud et al. (1954) report an Rf value of 1.6 [sic] on paper in phenol-ammonia, and that a faster-running yellow fraction was present if the chromatogram was run in the light. I have been able to chromatograph it on alumina by elution with ether, and a reddish-purple fraction which runs more rapidly than the main blue-violet band is then seen.

Absorption spectra. In ethanol solution there is a strong absorption band at about 580 mμ and an absorption minimum near 440 mμ (Lecoq de Boisbaudran, 1882; Scaffidi, 1913, 1914; Lasseur and Girardet, 1926; Gilman, 1953; Ehrismann and Noethling, 1936). In acetone these are at 570 and 445 mμ (Audebaud et al., 1954). The data of Waeldele (1938, p.111) and of Sartory, Meyer and Waeldele (1938a, 1938b) are in reasonabld agreement. Lasseur and Girardet report weak bands at 266, 280, and 630 mμ which I was not able to confirm. The accurate work of Ehrismann and Noethling on the crystalline violacein of Wrede and Rothhaas gave the following maxima and minima and specific extinctions (optical density of 1 cm thickness of a solution containing 1 g/litre) in ethyl alcohol:

mμ	265	334	366	436	579
	max.	min.	max.	min.	max.
Extinction	30.0	7.1	9.1	4.5	46.8

My own results have been close to these, but the specific extinctions were a little higher (Sneath, 1956b). DeMoss and Evans (1958) give data suggesting a specific extinction of about 44 at 565 mμ. Samples of violacein from strains RV (a mesophil) and NT (a psychrophil) were purified by the method of Strong (1944), and recrystallized from pyridine plus chloroform. The two pigments had identical absorption spectra in 96% ethanol, and the maxima and minima and specific extinctions of that from RV (which appeared slightly purer) were:

mμ	208	247	257.5	339
	max.	min.	max.	min.
Extinction	110	51.3	54.9	13.6

mμ	374	431	579
	max.	min.	max.
Extinction	17.8	7.4	65.4

The absorption spectrum was also examined in 10% (v/v) sulfuric acid (specific gravity 1.84) in 96% ethanol. The green solution was stable for some hours, and the maxima and minima and specific extinctions of pigment from RV were:

mμ	235 min.	250 max.	269 min.	280 max.	295 min.	301 max.
Extinction	40.4	52.2	36.8	41.6	29.1	29.5

mμ	337 min.	413 max.	502 min.	653 max.	659 min.	699 max.
Extinction	6.2	21.3	8.5	73.4	73.0	102.7

These data are in fair agreement with those of Lasseur and Girardet (1926) who found for similar solutions maxima at 266, 280, 587, 639, and 705 mμ.

The pigment from mesophils (strains MK and RV) and psychrophils (strains NT and HB) were compared with the reagents mentioned above, and by spectrophotometry. They behaved identically, except that with crude pigment there are impurities which obscure the absorption in the ultraviolet region. All of the strains of Chromobacterium given in Table 1 were tested to confirm that their violet pigment was nondiffusing, was soluble in ethanol, and became green and then red-brown with caustic potash. There seems, therefore, no reasonable doubt that the pigments of mesophils (C. violaceum) and psychrophils (C. lividum) are identical. To confirm this Dr. R.K. Callow kindly examined the crystalline samples of pigment from RV and NT by infrared spectrophotometry, and found them to be the same: the spectra were published in Sneath (1956b). X-ray crystallography also showed that they were identical (see Protocol 7 in Appendix I).

In Figs. 10 and 11 are shown absorption curves of crude and purified violacein, and those of some other bacterial pigments for comparison. These data are in good agreement with the curves given by Gilman (1953).

Purification of violacein. The poor solubility in all solvents makes it difficult to purify the crude pigment obtained by evaporation of alcohol or acetone extracts of bacteria. Tobie (1935) removed lipids by extraction with fat solvents. Friedheim (1932a) precipitated it from dry ether by passing in dry hydrogen chloride. Waeldele (1938, p.95) extracted the crude pigment successively with light petroleum, chloroform and water and crystallized it by slow evaporation from acetone; he obtained rosettes of fine needles. The best method is that of Strong (1944) who extracted lipids with dry chloroform and then with dry ether in a Soxhlet apparatus, and recrystallized the insoluble residue from hot pyridine by adding chloroform and dried in in vacuo at 80°. He obtained needles and rectangular plates. This was presumably the method used by Wrede and Rothhaas (1934a). I have used this method and find it essential to remove all ethanol from the chloroform and the ether by repeated extraction with water, or the pigment is dissolved.

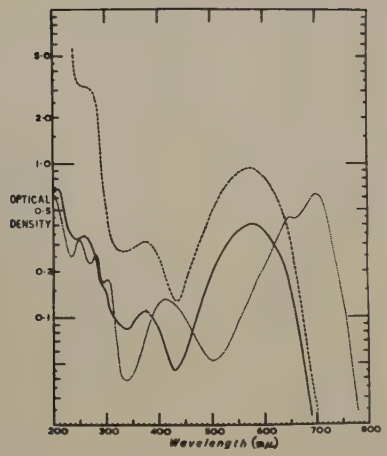


Figure 10a. Absorption spectrum of violacein (in $\frac{1}{2}$ cm cells).
——— = Ethanolic solution of crystalline violacein (14.0 mg/litre) from strain RV;
····· = crystalline violacein (14.0 mg/litre) from strain RV in 10 v/v sulfuric acid in 96% ethanol;
----- = crude violacein from strain HB in ethanol. (Sneath, 1956b)

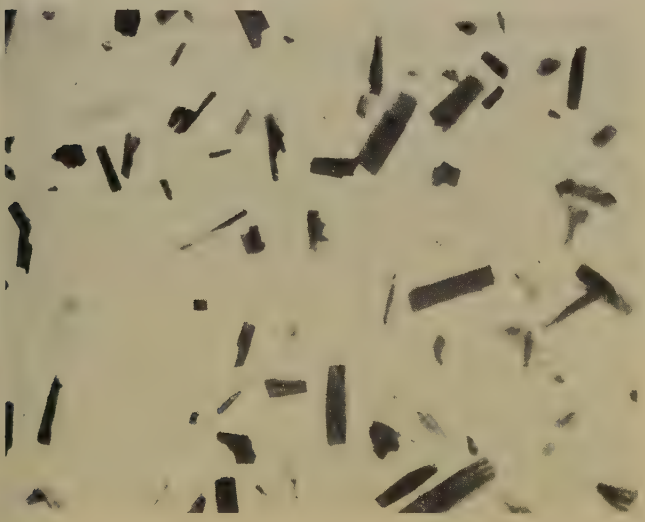


Figure 10b. Crystalline violacein from strain RV. x 100.

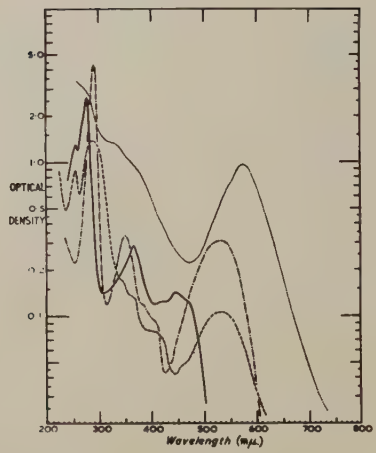
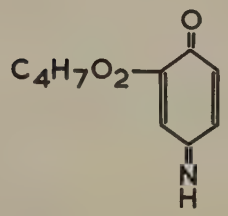


Figure 11. Absorption spectra of pigments of "Chromobacterium viscosum" strain GR (No. 25), "Chromobacterium iodinum" strain RE (No. 61) and "Chromobacterium ianthinum" strain TI (No. 59).
——— = pigment of TI in acid chloroform;
----- = pigment of TI in 0.1 M NaOH;
····· = pigment of GR in 0.5 M phosphate buffer pH 7.0;
-·-·- = iodinin in chloroform (9.4 mg/liter in ½ cm cell).
(From Sneath, 1956b)

Chemical structure.

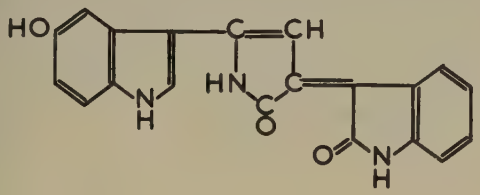
Reilly and Pyne (1927) reported the empirical formula to be about $C_{50}H_{59}O_{15}N_5$ with molecular weight c. 914. Kögl and Tönnis (see Kögl, 1932, p. 1443) crystallized it from acetone and reported it to be $C_{35}H_{25}O_6N_5$ or $C_{42}H_{30}O_7N_6$. Wrede and Rothhaas (1934a) and Wrede and Swane (1937) obtained it as small violet-black needles which decomposed without melting when heated. They report it as $C_{42}H_{28}O_7N_6$. It contained no O-methyl or N-methyl groups, on reduction took up 8 molecules of hydrogen to yield a colourless compound, and had no free carboxyl groups. It adds one equivalent of acids to yield salts, and forms derivatives with amines. The "hexaacetyl" derivative formed flat red needles. As noted by Tobie most of this work is of doubtful accuracy. He reported pyrrolic and indolic residues and iron (probably an impurity) in the molecule (Tobie, 1936, 1938, 1939).

Waeldele (1938, p. 108) and Sartory, Meyer and Waeldele (1938a, 1938b) suggested, without any good evidence, that violacein had a molecular weight of 180 with a structure:



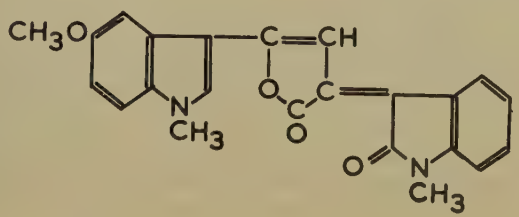
but this seems to be most unlikely, unless their organism (No.136) produced a different pigment.

Violacein has been studied in great detail by Beer and his colleagues (Beer et al., 1949, 1954; Ballantine et al., 1957a, 1957b, 1957c, 1958a, 1958b; Barrett et al., 1957). They found it yielded 5-hydroxyindole and oxindole on heating with zinc dust and at first favoured a hydroxyindolyl-oxindolyl-pyrryl-methene structure, which is reminiscent of the tripyrrylmethene structure of prodigiosin (see p. 298). Later, however, they favoured a structure 5-3(5 hydroxyindolyl)3-3(2 oxindolylidene) 2-oxopyrolline:

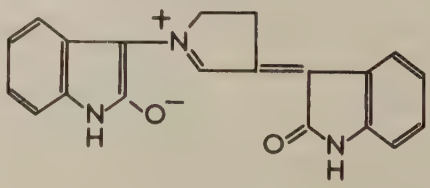


M.Wt.= 343·33

On reduction they obtained a pale yellow compound $C_{20}H_{16}O_5N_2$ which is γ(5-hydroxy-3-indolyl)-α-oxindolyl-γ-oxobutyric acid (M. Pt. 252-254° decomp.) and this yields a methyl ester (M.Pt. 247-249°) a tetramethyl derivative (M.Pt. 181°) a trimethyl derivative (M.Pt. 266-267° decomp.) and a red lactone (M.Pt. 286° decomp.). The trimethyl derivative yields a magenta-coloured lactone (M.Pt. 268-270° decomp.). These derivatives would serve to characterize violacein. The magenta lactone appears to be

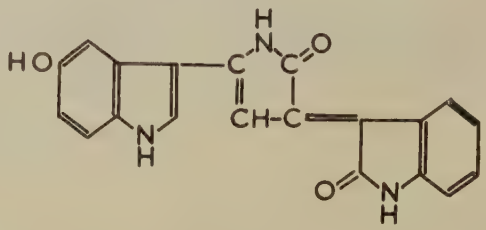


It is interesting to note the similarity of these compounds to the structure of isatin blue produced by condensation of isatin and pyrrolidine, which (Johnson and McCaldin, 1957) is probably



Barrett et al. (1957) showed that indolyl butyrolactams can condense with isatin to yield similar compounds, and it is tempting to think that violacein is formed by the condensation of 5 hydroxyindolyl butyrolactam with isatin. Ballantine et al. (1958b), by a brilliant piece of chemistry, have recently synthesized violacein and its tetramethyl derivative through the 5 methoxyindolyl-oxindolyl-oxopyrolline.

Ballantine et al. (1958b) believe that the trans arrangement of the pyrollone and oxindole nitrogen atoms is the most likely one in violacein: viz



VIOLACEIN: $C_{20}H_{13}O_3N_3$.

M.Wt. = 343·33.

Other pigments produced by Chromobacterium.

The reddish component in crude violacein and the yellow breakdown product have been mentioned. The latter may be the same as the pale yellow pigment which is produced by some pale variants (e.g. Martin, 1931) and which Corpe (1953) found to be increased by adding tryptophan to the medium. This pigment diffused somewhat. Ballantine et al. (1958b) report that a second pigment has been identified as desoxyviolacein (it is the indolyl instead of the 5-hydroxyindolyl compound) and this has been synthesized.

The function of violacein.

The work of Pfeffer (1896), Shibita (1912) and Ewart (1897) is inconclusive. Friedheim (1932a, 1932b) found that it did not yield up oxygen under anaerobic conditions, but he and Sartory et al. (1938a, 1938b) reported that violacein increased the respiratory coefficient of nonpigmented variants of Chromobacterium. Its function, if any, remains obscure. It may protect the bacteria from predatory protozoa (see below).

Cultural conditions for pigment production.

Like Serratia, Chromobacterium only produces pigment in the presence of abundant oxygen. Sullivan (1905) found that traces of magnesium, sulfate, and phosphate were essential for pigmentation, and that acid media were unsuitable. Sartory et al. (1938a, 1938b) found iron to be essential, and that carbohydrates had little influence. DeMoss and Happel (1955) say that tryptophan is needed, but it is certainly not essential though it may be stimulatory. Lasseur and Girardet (1926) found good pigmentation on asparagine-salts medium, and histidine or proline can replace asparagine (Marchal and Baldo, 1956). Pigmentation is best at temperatures a little below the optimum for growth (Bampton, 1913). Minett (1913) and Oliver (1902) found light to increase pigmentation: I have noticed no effect. Pigmentation is inhibited by phenol (Galeotti, 1892), carbon dioxide (Shibita, 1912) and subinhibitory concentrations of chloromycetin, tetracyclins and streptomycin, but stimulated by low concentrations of penicillin, neomycin, and framycetin (Darrasse et al., 1955). Koch (1938) and Corpe (1953) found yeast extract and some meat extracts to be inhibitory. Bortels (1952) suggests, but on quite inadequate evidence, that meteorological factors may markedly affect pigmentation.

The antibiotic activity.

Matruchot (1898, 1900) had shown that violacein was absorbed by fungal mycelium. Shahan (1943) noted that Chromobacterium violaceum had an antibacterial effect on other bacteria, and Lichstein and Van de Sand (1945, 1946) found it was most active against Gram-positive organisms (also Neisseria) and some yeasts, and poorly active against Gram-negative bacteria, Clostridium welchii and fungi. A concentration of 0.001% was bacteriocidal to Staphylococcus aureus. It was of low toxicity to mice and did not inhibit phagocytosis, but unfortunately the antibiotic activity was inhibited by serum, and in pneumococcal infections in mice it only delayed death. Müse and Pötsch (1954) reported similar findings. They found that staphylococci, streptococci, Sarcina, Bacillus, Corynebacterium and Candida were sensitive to the pigment, and, to a lesser degree, Pseudomonas and Serratia, while coli-typhoid-dysentery organisms, Proteus and Brucella were resistant. They noted that the activity was not destroyed by ultraviolet light or dry heat at 180° for 3 hours or moist heat at 100° for 1 hour.

Violacein is toxic to soil amoebae (Singh, 1942, 1945) and the bacterium is toxic on ingestion to the ciliates Colpoda and Colpidium, probably because of the pigment (Kidder and Stuart, 1939; Burbanck, 1942).

Biosynthesis of violacein.

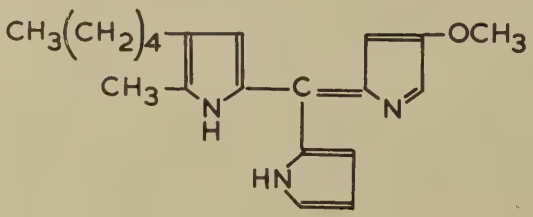
Little work has been done on the biosynthesis of violacein. DeMoss and Happel (1955, 1956) observed that tryptophan increased pigmentation, while oxindole, 5-hydroxytryptophan and indole-3-acetic acid, indole, anthranilic acid and tyrosine did not. However, Mitoma et al. (1955, 1956) have shown that Chromobacterium converts tryptophan to 5-hydroxytryptophan and it is likely that this is the source of the 5-hydroxyindolyl moiety of violacein. DeMoss and Happel used C^{14} labelled L-tryptophan (not D-tryptophan as printed: DeMoss personal communication), and found that the indole skeleton is incorporated and also the α carbon atom. A number of other substrates, including bicarbonate, acetate, lactate, succinate, ribose, glucose, glycine, alanine and lysine were not incorporated into the violacein. They noted two fractions in the crude pigment on paper chromatography, and also that nonpigmented variants produce colourless intermediate compounds which can be converted to violacein by pigmented cells or apparently even nonenzymically. The position is thus similar to that in Serratia (see Santer and Vogel, 1956; Green and Williams, 1957). DeMoss and Evans (1958) found that two molecules of tryptophan appeared to yield one molecule of violacein, but a second substance was also incorporated into the pigment. They found that the 3-C atom of serine was incorporated (presumably into the pyrollone ring). They noted that though the α, β, and ring carbon atoms of tryptophan were incorporated into violacein, the carboxyl carbon atom was not.

Other bacterial pigments

There are a number of other blue or violet pigments which may be confused with violacein, and these are briefly reviewed here, with a few comments on prodigiosin, which resembles violacein in many of its properties.

Prodigiosin.

This red pigment from Serratia marcescens (syn. Chromobacterium prodigiosum Topley and Wilson 1929) has the following structure according to Wrede and Rothhaas (1934b)—though this should be re-examined in view of recent work on violacein:



It is insoluble in water but is soluble in chloroform and ethanol. Enrismann and Noethling (1936), Gorbach (1930) and Hubbard and Rimington

(1950) have published spectral absorption curves which differ considerably, perhaps because they are sensitive to pH changes or because the samples were impure. Morgan and Tanner (1955) report the infrared spectrum. They found the free base to have M.Pt. 151-152° and the perchlorate M.Pt. 227-229°. In acid and alkaline ethanol the absorption maxima were 541 and 468.5 mμ respectively. Some X-ray crystallographic data are given in Protocol 7. Amako (1930), Weiss (1949) and Williams and his colleagues (Williams, Green and Rappaport, 1956; Green, Rappaport and Williams, 1956) found several components in crude pigment. The latter authors found orange, blue and minor red fractions as well as the main red fraction.

Indigoidine.

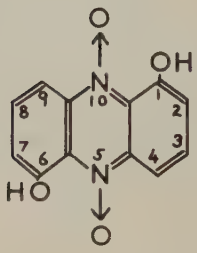
This blue pigment is formed by the "indigo bacteria," (Pseudomonas indigofera and its allies, Nos. 34-39 in Appendix II), and most of our knowledge is given by Elazari-Volcani (1939, 1954). It is insoluble in water, ethanol, benzene and chloroform, and slightly soluble in phenol, aniline, quinoline, nitrobenzene and pyridine. The last dissolves 0.2 mg/ml. It gives a blue solution in strong hydrochloric acid (turning brown), brown solutions in strong sulfuric acid and nitric acid, and in caustic potash a blue solution which turns yellow. It is reduced by sodium hydrosulfite to give a colourless solution, which becomes blue on aeration. These reactions are in good agreement with those reported by Claessen (1890) and Schneider (1894).

Elazeri-Volcani (1939) extracted the bacteria with hot chloroform and then with hot ethanol to remove lipids, and then extracted the pigment with hot pyridine and evaporated this in vacuo. It was not crystallized. It gave an acetyl derivative (with pyridine and acetic anhydride) which gave orange red needles from nitrobenzene (M.Pt. over 300° decomp.). The benzoyl derivative formed dark red needles (M.Pt. over 280° decomp.) from chloroform-carbon tetrachloride. Both the acetyl and benzoyl derivatives reformed the original pigment on hydrolysis. Indigoidine in pyridine shows maximal absorption at 605 mμ: it is not indigo (maximum at 614 mμ, stable in strong acids).

The pigment of Corynebacterium insidiosum (No. 43), of strains of Erwinia chrysanthemi and of a strain of Arthrobacter is identical with indigoidine (Starr, 1955, 1958; Kuhn and Starr, 1956). It is uncertain whether the pigment of Pseudomonas lemonnieri (No. 31) is the same.

Iodinin.

This violet pigment from Chromobacterium iodinum Davis (No. 61) is 1:6 dihydroxyphenazine 5:10 dioxide (International nomenclature) (Clemo and McIlwain, 1938; Clemo and Daglish, 1948, 1950; Yosoika and Kidani, 1952a, 1952b; Pachter and Kloetzel, 1952; Serebryanyi, 1952):



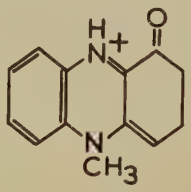
It is unusual in being an N-oxide. Phenazine compounds are uncommon in nature: the best known are pyocyanin, chlororaphin and the pigment of Pseudomonas aureofaciens (see p. 301). Iodinin is soluble in chloroform, in alkalies (giving a green solution), slightly in ethanol but not in water or dilute acids. It crystallizes as needles (M.Pt. 236° decomp.). The red-violet solution in chloroform shows the following absorption maxima and minima and Molecular Extinction Coefficients (Sneath, 1956b).

mμ	255	291	317	352	427	534
	min.	max.	min.	max.	min.	max.
Extinction	7,270	140,000	3,900	11,000	1,350	10,100

The spectrum is shown in Fig. 11. Some X-ray crystallographic data are given in Protocol 7. The infrared spectrum is given by Clemo and Daglish (1950). On reduction it gives 1:6 dihydroxyphenazine (orange needles M.Pt. 274°, dimethyl derivative M.Pt. 247°, diacetyl derivative M.Pt. 233°). It has some antibiotic activity (McIlwain, 1943a, 1943b; Wiedling, 1945; Frisk, 1946; Iland, 1948) mainly against Gram-positive bacteria, including the tubercle bacillus. Witkop and Kissman (1953) suggest that iodinin may arise from the rearrangement of an unstable peroxide, attributing this theory to Clemo and Daglish (1950).

Pyocyanin.

The blue phenazine pigment of Pseudomonas aeruginosa is soluble in water, ethanol and chloroform. Wrede and Strack (1929) believed it was a dimer, but Kuhn and Schön (1935) believe it is an ionized monomer



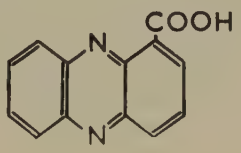
Ehrismann and Noethling (1936) give the absorption spectrum and found these Molecular Extinctions:

mμ	660 max.	460 min.	326 max.	281 min.
Extinction	2,900	340	13,000	630

Some X-ray crystallographic data are given in Protocol 7. The yellow fluorescent pigment of this bacterium (fluorescein or pyoverdin) is still little known (see Hugo and Turner, 1957; Elliott, 1958). Pyocyanin has also been reported to be produced by a coccus (Färber, 1951 and see No. 51a in Appendix II).

Chlororaphin.

This green phenazine pigment from Pseudomonas chlororaphis is a reduced dimer of phenazine-1-carboxamide (Kögl and Postowsky, 1930). The oxidized form (oxychlororaphin) is yellow, and is phenazine-1-carboxamide itself. Pseudomonas aureofaciens Kluyver, 1956 produces an orange pigment which is the corresponding free acid (Haynes et al., 1956 and see Protocol 7):



Recently, Morris and Roberts (1959) reported that the purple pigment of the unnamed pseudomonads of the same type as No. 59 in Appendix II is a phenazine compound, though its full structure has not yet been elucidated. When the acid form of this pigment is crystallized from benzene-cyclohexane it forms orange-yellow needles (M.Pt. 237-238°). Further details are given in Appendix II.

Pigment of Bacillus le Monnieri (No. 31 in Appendix II).

This blue pigment is described by Lasseur and Girardet (1926). It is insoluble in water, ethanol and fat solvents, but is soluble in pyridine and acids in which it gives a blue solution turning green in air. The distinction from indigoidine is not certain, and it seems very similar in properties. However, Lasseur and Girardet report that in pyridine it has an absorption maximum at 630 mμ, while indigoidine shows it at 605 mμ. Hugo and Turner (1957) give a spectrum in dimethylformamide.

Caryocyanin.

The violet pigment from Pseudomonas caryocyanea (No. 28 in Appendix II) has been studied by Dupaix (1933, p. 125 et seq.). It is soluble in water, becomes green with acids and blue (unstable) with alkalies. In neutral aqueous solution it shows maximal absorption at 588.5 mμ. It is very similar to the pigment of Bacillus cyaneo-fluorescens (No. 30).

Viscosin.

The blue water-soluble pigment of Chromobacterium viscosum Grimes is described with that organism in Appendix II. It seems very similar to a number of pigments produced by a group of bacteria described in Appendix II as Nos. 17-26. Its constitution is unknown. At pH 7.0 it shows maximal absorption at 574 mμ.

CHAPTER XI

CARBOHYDRATE FERMENTATION REACTIONS

As noted previously there is general agreement that Chromobacterium does not produce gas from carbohydrates. Reports on the production of acid are confused and contradictory, probably because of the different techniques which were used, since these bacteria produce little acidity, and psychrophils in particular only produce it aerobically. Thus Grimes (1930) found no acidity with any carbohydrate, while Ward (1898) found slight acidity in glucose, and Dodd (1941, pp. 24-25) reports alkalinity in glucose. It is evident that reports which do not specify the technique which was employed are of little value.

There is general agreement that in the usual peptone-water carbohydrate media there is no definite acidity with mannitol, dulcitol, raffinose, xylose, arabinose, sorbitol, glycerol, inositol, rhamnose, or adonitol with either mesophilic or psychrophilic strains, though as shown below acidity can be shown with some of these by using special media. Cellobiose is not acidified (Tittsler and Sandholzer, 1936). Fermi (1890) and Grimes (1930) reported strains which hydrolyzed starch slowly. The main reports on differential carbohydrate reactions are summarized in Table 5.

It will be seen that mesophils (i.e. C. violaceum) usually produce acid from glucose, fructose, trehalose and mannose and often from sucrose, dextrin, and starch. Psychrophils (i.e. C. lividum) give very variable reactions. The results obtained with the strains in Table 1 are given in Protocol 8 in Appendix I, using peptone water media, and are summarized in Table 6.

Most of these carbohydrates were tested with mesophils at 37° also, and they gave the same reactions. It is seen that trehalose is the carbohydrate which most sharply divides the mesophils from the psychrophils. The reactions with starch, dextrin and glycogen were always very similar, and were delayed for 7 or more days: presumably this was due to the appearance of mutants possessing an amylase, and from one of the tubes of BN a variant was obtained which fermented starch promptly, unlike the parent, by plating onto a plate containing starch and an indicator.

The subdivision of mesophils on the reactions with sucrose and starch does not coincide with any other features and they do not therefore seem to be distinct subspecies. Similarly, the weak reactions given by psychrophils are not practicable for subdividing them. As noted below, aesculin also differentiates mesophils from psychrophils, but as the latter produce such weak acidity it must be used with an iron indicator (see p. 309).

Table 5. Differential carbohydrate reactions recorded in the literature, other than fragmentary data, and presumably all tested in broth or peptone water media.

Carbohydrate	Authors*												
	1	2	3	4	5	6	7	8	9	10	1	10	11
	Mesophils										Psychrophils		
Glucose	A	A	A	A	A	A	A	A	A	A	(a)	(A)	A
Fructose	A	A	A				A	A	A		(a)		
Sucrose	(A1)	(A)	AG	-	(A)	(A)	-	A1	-	-	-	(a)	(a)
Maltose	(?)	-	-	-	(A)	(a)	-	A	-	-	(?)	(A)	(a)
Lactose	-	-	-	-	(A)	-	-	?	-	-	-	-	-
Salicin	(?)	-	-	-		(a)		-	-		(?)		
Dextrin	(A)	(a)					-	-	-		-		
Starch	(A)		-								-		
Trehalose	A	A	AG								-		
Galactose	-	-	-			-	A	-	-		(?)		
Inulin	(?)	(a)	-			-	-	-	-		(?)		
Mannose	(A)	A									(a)		
Aesculin	-	-	-								(?)		

Symbols: A = marked acidity, a = slight acidity, ? = doubtful acidity, - = no acidity, G = gas, l = late acidity. Symbols in brackets indicate that only some strains produced acidity. Blank = not recorded.

* 1 = present study and Sneath (1956b). 2 = Sippel et al. (1954) and Sippel (1955, p. 40). 3 = Audebaud et al. (1954). 4 = Gilman (1953). 5 = Morris (1954). 6 = Cunningham and Raghavachari (1924). 7 = Minett (1913). 8 = Lesslar (1927). 9 = Ramchandani (1930). 10 = Cruess-Callaghan and Gorman (1935). 11 = Bampton (1913).

Table 6. Carbohydrate fermentation reactions in 1% carbohydrate in peptone water at 25° with strains in Table 1.

Number of strains giving the pattern	Carbohydrate					
	Glucose	Fructose	Mannose	Trehalose	Sucrose	Starch Dextrin & Glycogen
			Mesophils (C. violaceum)			
2	+	+	+	+	+	+
3	+	+	+	+	+	-
3	+	+	+	+	-	+
10	+	+	+	+	-	-
			Psychrophils (C. lividum)			
2	+	+	+	-	-	-
1	+	+	-	-	-	-
2	+	-	-	-	-	-
3	-	+	-	-	-	-
12	-	-	-	-	-	-

Symbols: + = definite acid production within 14 days.
- = doubtful or negative acidity.
Some reactions were delayed for 7 to 14 days (see Protocol 8 in Appendix I), and with psychrophils they were usually weak.

The following carbohydrates showed no definite acidity with any strain: Cellobiose, raffinose, melezitose, m-erythritol, dulcitol, inositol, adonitol, lactose, rhamnose, α-melibiose.

The following sometimes gave doubtful but inconstant reactions: Salicin, D(+)xylose, L(+)arabinose, mannitol, aesculin, inulin, galactose, sorbose, glycerol, sorbitol, and maltose.

No gas was produced in any instance.

Carbohydrate reactions in special media

Corpe (1954) used an agar medium containing ammonium salts, carbohydrate and indicator of acid (but not further described) and inoculated the surface. He found much variation between strains. Three mesophilic strains acidified glucose, trehalose and sometimes fructose and galactose. Twenty-one psychrophils were tested: all acidified glucose and most of them also acidified maltose and trehalose. A few strains acidified one or more of a wide range of carbohydrates. These results are not in agreement with those of Leifson (1956b) or myself, even with strains examined by all three workers.

Leifson (1956a, 1956b) used the sensitive medium of Hugh and Leifson (1953). In this the peptone concentration is reduced and it is made semi-solid with agar. Acidity in the depths as well as at the surface indicates anaerobic (fermentative) attack on the carbohydrate. Acidity at the surface only indicates aerobic (oxidative) attack. I have also examined my strains in this medium (see Protocol 9 in Appendix I). Our results are in good agreement, and may be summarized as follows.

Typical mesophils.

The majority of mesophils produce acid from carbohydrates fermentatively. We both find acid from glucose, fructose, and mannose, and sometimes sucrose, but not from lactose, arabinose, xylose, mannitol, dulcitol, or salicin. I find acid also from trehalose, not from galactose, m-inositol, or cellobiose, but sometimes from starch. Leifson found acid from inulin (which seems to be caused by decomposition of inulin by heat—see below) and often from maltose, but I found these negative or doubtful. Leifson reports no acid from sorbitol, while I find that there is usually detectable acid. Glycerol occasionally shows slight acidity, but only aerobically (see Protocol 9). Leifson calls this group Chromobacterium manilae (No. 81). Strain BM (see Table 2) was typical in fermenting glucose, fructose, mannose, and trehalose.

Atypical mesophils.

Leifson calls these Chromobacterium laurentium (No. 80). They attack carbohydrates only aerobically. Leifson found them to attack only glucose and fructose. I find that trehalose is also oxidized and sometimes mannose and glycerol. The other carbohydrates mentioned above are not attacked.

Psychrophils.

These attack carbohydrates only aerobically. We both find them to oxidize glucose, fructose, mannose, arabinose, xylose, mannitol, and usually sorbitol, sucrose, and maltose, and to have no action on dulcitol. Leifson finds inulin, salicin, and lactose are not attacked: a few of my strains attack the first two, and all attack lactose, but only after considerable delay. I find that they also acidify m-inositol and usually galactose, glycerol, and cellobiose, but not trehalose and starch.

It is evident that the pattern of reactions of mesophils and psychrophils is quite different. The former attack a few carbohydrates energetically, the latter attack a wide range but less actively. The reactions in

Hugh and Leifson media which best distinguish the groups are shown below.

Carbohydrate	Mesophils (C. violaceum)	Psychrophils (C. lividum)
Trehalose	+	-
Arabinose	-	+
Xylose	-	+
Lactose	-	+ (late)
m-inositol	-	+
Mannitol	-	+
Cellobiose	-	Usually +
Glycerol	Usually -	Usually +
Galactose	-	Usually +

Eltinge (1957) uses maltose to differentiate the groups, but there are many doubtful reactions with mesophils in my hands.

Anomalous results with inulin.

Leifson (1956b) found mesophils to attack inulin, while I did not. It seemed likely that the sensitivity of inulin to breakdown by heat was responsible, and on comparing (in Hugh and Leifson medium) inulin which had been sterilized by heating at 115° for 15 minutes with inulin which was Seitz-filtered it was found that all fermentative (typical) mesophils produced acid anaerobically from heated inulin but not from filtered inulin. The atypical (oxidative) mesophils attacked neither, so the breakdown product is evidently not fructose.

Aberrant strains.

It seems likely that the atypical mesophils (strains SH, LW, RT, and MH) are loss-variants. Probably the strain EF (see Table 2) is also a loss-variant; it would not produce acid from glucose or any other carbohydrate either aerobically or anaerobically. Strain ED oxidized arabinose, xylose, mannitol, and sucrose and was doubtful with trehalose. Strain BC was atypical in not attacking mannitol.

CHAPTER XII

BIOCHEMICAL TESTS

The results found with the strains of Chromobacterium which were studied are shown in detail in Protocols 10 to 16 in Appendix I. Most of the data are from Sneath (1956b) where technical details are given in full.

Litmus milk. Almost all possible reactions have been reported by one author or another. Thus Germano (1892), Ward (1898), and Sippel (1955, p. 40) reported alkalinity with slow peptonization and variable clotting. Cruess-Callaghan and Gorman (1935) found no clot, but sometimes bleaching of the litmus. Bampton (1913) found wide variation in peptonization and clotting. Woolley (1904, 1905), Boyce and Hill (1900),

Minett (1913), Lesslar (1927) and Morris (1954) observed acidity, usually slight, with a slight clot and slight peptonization. A violet ring or pellicle is usually seen. Corpe (1954) found that psychrophils reduced the litmus.

The results are very variable in my experience (Protocol 10). There may be slight acidity, more common among mesophils, which may later revert to alkalinity. There may be marked alkalinity, seen in both groups. The mesophils usually peptonize the casein and may give a small clot which is later digested. The psychrophils often produce a large firm "rennin" clot, and some strains bleach the litmus; there is seldom much peptonization in 14 days. A violet ring or pellicle is common. The most useful feature is peptonization but this is easier to test in casein plates (see p. 309).

Indole. Most authors have found strains of Chromobacterium to be indole-negative (e.g. Bampton, 1913; Grimes, 1930; Cruess-Callaghan and Gorman, 1935; Corpe, 1954; Leifson, 1956b). There are a few reports of positive reactions (Lehmann and Neumann, 1899, Vol. 2, p. 263, but see Table following p. 495; Dyar, 1895; Morris, 1897; Calderini, 1925; Ramchandani, 1930) but some of these workers mention that they used the nitroso-indole reaction, which is not very reliable. The only positive results using the modern p-aminobenzaldehyde method are those reported by Waeldele (1938, p. 80) and by Wilson and Miles (1946, Vol. 1, p. 634). In my hands all strains are indole-negative.

Ammonia production. Ammonia is produced from proteins and the NH_3 test is positive (Marchal, 1893a, 1893b; Martin, 1931; Wilson and Miles, 1946, Vol. 1, p. 634; Sneath et al., 1953). Aubel and Colin (1914) found that glucose inhibits the formation of ammonia from asparagine. In my experience all strains produce ammonia in peptone water, but some only weakly.

Methyl Red and Voges-Proskauer tests. These tests are both negative or only doubtfully positive (Sippel, 1955, p. 40; Audebaud et al., 1954; Sneath et al., 1953; Wilson and Miles, 1946, Vol. 1, p. 634; Cunningham and Raghavachari, 1924). I have found that a few mesophils gave doubtful Methyl Red reactions; the remainder and all psychrophils were M. R. negative. All strains were V.P. negative, (the few doubtful reactions appeared to be due to the effect of alkali upon the pigment, which became green and then brown).

H_2S production. This test gives variable results. Stagnitta-Balistreri (1893), Morris (1897), Lehmann and Neumann (1899, Vol. 2, Table following p. 495, but see also p. 263), Corpe (1954), Audebaud et al. (1954), Sippel et al. (1954), Gilman (1953), and Darrasse et al. (1955) found the reaction to be negative. Bampton (1913) found a few strains to be positive. and Floch and de Lajudie (1943), Wilson and Miles (1946, Vol. 1, p. 634) and Sneath et al. (1953) found weak or doubtful positive reactions. At any rate Chromobacterium does not produce abundant H_2S from peptone, and I found only a few doubtful reactions with some mesophilic strains.

Methylene blue reduction. Martin (1931) found this test to be negative, as did Audebaud et al. (1954). Gilman (1953) reported it as weakly positive and Wilson and Miles (1946, Vol. 1, p. 634) as positive. Müller (1899) found the pigment to interfere with the reading of the test. Strains of Chromobacterium did not reduce neutral red (Minett, 1913; Deshusses and Novel, 1939). However, Waeldele (1938, p. 80) reports a strain which

did reduce it. Dyar (1895) found no reduction of rosolic acid, while Wright (1895) reported the colour to be deepened. Chromobacterium evidently does not produce a very low Eh potential in culture. Most of the mesophils I have tested showed definite reduction of methylene-blue, while most psychrophils showed no reduction. However, there were some doubtful results with strains of both groups.

Reduction of nitrates. Frankland and Frankland (1889) first observed that strains of Chromobacterium reduce nitrate to nitrite, and most subsequent authors agree that the majority of strains can do so. Calderini (1925), Grimes (1930), and Corpe (1954) found that all of their numerous strains did so, and Grimes named two new species of Chromobacterium on this account (Nos. 93 and 114), because Bergey et al. (1926, p. 123) reported, erroneously, that members of the genus could not reduce nitrate. Cruess-Callaghan and Gorman (1935) found mesophilic strains were negative or weakly positive, but it is likely that this was because these strains had also destroyed the nitrite which they formed (see below). However, Leifson (1956b), Eltinge (1956) and myself have found occasional strains which are unable to reduce nitrate at all. Eltinge (1956, 1957) classifies the strains into species on the reactions with nitrate (see p. 251), but this is a most unsuitable basis for the taxonomy, especially as she does not give the exact experimental conditions which are to be considered decisive. The majority of strains listed in Table 1 reduced nitrates, usually after one day. A few, in particular strain TV, destroyed all the nitrate and nitrite after 5 days, so that the test for nitrite became negative.

Destruction of nitrite. Corpe (1954) found that most psychrophilic strains would produce bubbles of nitrogen gas from nitrates, and Eltinge (1956) confirmed this. In contrast, mesophils do not show visible gas, although they can also generally destroy nitrite (Sneath, 1956b). In that paper I reported that visible gas was not produced by psychrophils, but further work shows that some psychrophils can do so, though this is greatly affected by the composition of the medium used. In the medium for testing destruction of nitrite used by Sneath (1956b) most strains of both groups destroyed it readily. In the medium of ZoBell (1932) eight out of twenty psychrophils produced bubbles of gas. Eltinge (1956, 1957) reports that psychrophils destroy nitrites "completely" (under conditions which are not precisely described), or at any rate more rapidly than mesophils, but I find that this is not always so.

One variant of strain SH was obtained which was unable to reduce nitrate or to destroy nitrite, and there are two other strains which are unable to carry out either process. This suggests the loss of a factor necessary to both processes, and presumably these strains are loss-variants.

Nitrogen fixation. This does not seem to have been studied. Gray and Smith (1950) and Anderson (1955) have observed yellow chromogens which fix nitrogen which may perhaps be related to psychrophilic chromobacteria.

Catalase. This is positive, though rather weakly so (Grimes, 1930; Martin, 1931; Floch and de Lajudie, 1943; Gilman, 1953; Audebaud et al. 1954; Sneath et al., 1953). I found all my strains to be weakly positive, and pouring hydrogen peroxide onto a culture often gave doubtful results,

but if the growth was emulsified in the hydrogen peroxide solution bubbles of gas were clearly seen.

Urease. These organisms do not produce urease (Audebaud et al., 1954; Darrasse et al., 1955; Leifson, 1956b). All my strains were urease-negative.

Phosphatase. Bray and King (1943) found a strain to be positive. Leahy, Sandholzer and Woodside (1939) also demonstrated phosphatase in the cells. All my strains were moderately or strongly positive by the Bray and King technique.

Hydrolysis of aesculin. This test generally distinguishes mesophils from psychrophils. With the strains listed in Table 1 all the psychrophils hydrolyzed it, as shown by a dark brown colour in the presence of iron as an indicator of hydrolysis. None of the mesophils hydrolyzed it within 14 days in liquid medium or within 4 days on plates. The liquid and solid media gave concordant results, but reactions appeared more quickly on the latter. Since then the strains listed in Table 2 have been tested, and two psychrophilic strains which do not hydrolyze aesculin (strains ED and EF) have been found.

Hydrolysis of casein. Zones of clearing in milk plates due to hydrolysis of casein were produced in 2 days at 25° by most mesophilic strains, but not by most psychrophils. After a few more days many psychrophils showed clearing beneath or close to the growth. The clearing parallels the peptonization of litmus milk.

Hydrolysis of gelatin. In plates containing gelatin in nutrient agar there was a wide zone of digestion after 4 days with most mesophils, and none (or digestion only beneath the growth) with psychrophils. This runs parallel to gelatin liquefaction in stab cultures.

Liquefaction of coagulated serum. Most mesophils liquefy Löffler's serum slopes after 14 days at 25° (see page 278); psychrophils show little or no liquefaction (see Protocol 13).

Hydrolysis of starch. Fermi (1890) and Mehta (1925) found weak diastatic activity. Grimes (1930) and Corpe (1953) found most strains to have no activity, but a few were positive. None of my strains hydrolyzed starch rapidly, though there was partial hydrolysis by strains PT and LG.

Hydrolysis on horse blood agar. As mentioned in Chapter VI, mesophils often produce some haemolysis. Most of the mesophils gave wide zones of partial clearing with a diffuse edge in 2 days at 25°. Psychrophils showed none, or only a trace, although after 4 days a few psychrophils gave definite slight haemolysis.

Egg yolk reaction. On plates containing egg yolk all the mesophils produced opaque zones in 2 days, and none of the psychrophils showed any after 4 days. This is presumably due to a lecithinase, which may be largely responsible for the haemolytic activity. The zones may show concentric markings.

Citrate utilization. All strains utilize citrate (pp. 286, 289).

Malonate utilization. The only strain which utilized malonate detectably in 2 days was strain CA. Several colonies of this strain were retested and they all utilized malonate. It is possible that with longer incubation other strains might be positive.

Production of phenylpyruvic acid from phenylalanine. All the strains were negative.

Table 7. Biochemical reactions of Chromobacterium.

Test	Mesophils (C. violaceum)	Psychrophils (C. lividum)
Litmus milk	Reaction variable: litmus not bleached: peptonization marked: clot small or none.	Reaction variable: litmus often bleached: peptonization slight: clot often bulky.
Indole	-	-
Ammonia	+	+
Methyl Red	doubtful or -	-
Voges-Proskauer	-	-
H_2S	doubtful or -	-
Methylene blue reduction	usually +	doubtful or -
Reduction of nitrate	usually +	usually +
Destruction of nitrite	usually +	usually +
Gas from nitrate	-	sometimes +
Catalase	+	+
Urease	-	-
Phosphatase	+	+
Hydrolysis of aesculin	-	usually +
Hydrolysis of casein	strong	weak
Hydrolysis of gelatin	strong	weak
Liquefaction of serum	strong	weak
Hydrolysis of starch	weak or -	-
Haemolysis on horse blood agar	strong	weak or -
Egg yolk reaction	+	-
Citrate utilization	often slow, +	prompt, +
Malonate utilization	-	usually -
Phenylpyruvate	-	-
Gluconate	doubtful or -	often +
Chitin digestion	usually +	usually -
Aryl sulfatase	-	-
HCN production	+	-

Gluconate test. This test is not specific for Pseudomonas, as was shown by Shaw and Clarke (1955). Using their medium, several strains of psychrophils produced from D-gluconic acid a substance which reduced Benedict's solution even in the cold (unlike 2-keto-D-gluconic acid). It was found that this substance will reduce phenol blue (E'_0 = + 0.224 V.) but not methylene blue (E'_0 = +0.011 V.) at pH 7.0, which suggests that it may be an analogue of ascorbic acid.

Digestion of chitin. Veldkamp (1955) and Clarke and Tracey (1956) reported that strains of Chromobacterium could digest chitin. I found that all mesophils (except strain FH) produced clear halos of digestion in 7 to 14 days, and only one of the psychrophils (strain BC) did so, using the technique described in Protocol 16.

Arylsulfatase. All strains were negative.

Summary. The biochemical reactions are summarized in Table 7. including the production of HCN (see below).

It is seen that there are many differences between mesophils (C. violaceum) and psychrophils (C. lividum). The mesophils possess much greater proteolytic activity, which is more or less parallel with the several substrates (casein, gelatin, serum) and possess more haemolytic and chitinolytic activity than psychrophils. The psychrophils show weaker activity in most tests, but as noted on page 305, they attack a much wider range of carbohydrates than mesophils.

CHAPTER XIII

PRODUCTS OF METABOLISM

Other than the pigment violacein, little is known about metabolic products of Chromobacterium. The production of ammonia from peptone and of various acids from glucose has already been mentioned. Like other bacteria, these organisms secrete vitamins during growth (Tittsler and Earle, 1941). They do not secrete polypeptide antibiotics like those of Serratia (Fuller and Horton, 1950). Bréaudat (1906) reported the production of small amounts of acetone by a strain (No. 89 in Appendix II) during growth on peptone (especially if sucrose and calcium carbonate were added), together with a little ethanol and some volatile acids. Beijerinck and Folpmers (1916) found chromobacteria to oxidize L-malate and fumarate to pyruvate.

Hydrogen cyanide. The production of hydrogen cyanide by mesophilic strains of Chromobacterium was reported by Sneath et al. (1953). This had been noted earlier by Clawson and Young (1913), and possibly by Hartley (1913) (whose strain (organism No. 76 in Appendix II) may have been a strain of Chromobacterium which produced a colour reaction with picric acid similar to that given by cyanide). Sippel (1955, p. 47) confirmed the production of hydrogen cyanide and found aerobic broth cultures to contain as much as 7.6 μg/ml of HCN. The production of cyanide by bacteria seems to be uncommon. Emerson, Cady and Bailey (1913), Patty (1921), and Lorck (1948) found that Pseudomonas aeruginosa produces small traces; it is only produced under aerobic conditions in neutral or acid media.

Most mesophilic strains of Chromobacterium produce enough hydrogen cyanide to be detected by smell when a Petri dish with a heavy overnight growth is first opened. Proof that it was hydrogen cyanide was obtained as follows: a confluent culture of strain MK on 200 ml of nutrient agar in a rubber-stoppered Roux bottle was incubated at 30° for 2 days and the enclosed air was then slowly blown through 1 ml of 10% aqueous NaOH mixed with 0.1 ml of 1% ferrous sulfate ($FeSO_4 \cdot 7H_2O$). After 10 minutes this solution was heated at 100° for a few seconds and a drop of 1% ferric chloride added. On cautious acidification with hydrochloric acid the colour of Prussian blue appeared.

A more sensitive test is the following: Nutrient or blood agar plates heavily inoculated and incubated at 25° for 2 days aerobically are tested by introducing the end of an indicator paper (not touching the medium) and replacing the lid. The indicator paper is made as follows: benzidine acetate is dissolved in boiling water and the saturated solution is cooled and filtered. To 10 ml of this is added 1 ml of 3% aqueous cupric acetate. A strip of filter paper is dipped in the freshly-prepared mixture, and this becomes blue in the presence of hydrogen cyanide. Chlorine, bromine, and hydrogen chloride also give a positive result (but can be excluded from consideration here) while hydrogen sulfide and sulfur dioxide inhibit the reaction (Anonymous, 1938). The paper may turn brown after about 10 minutes, apparently because of the ammonia from the cultures. By this method all the mesophilic strains gave a positive reaction for cyanide. It was noted that in anaerobic plate cultures cyanide could not be detected. None of the psychrophils gave a positive reaction, and strain HB did not yield a positive Prussian blue reaction when tested as described above on a Roux bottle. The two strains of Pseudomonas aeruginosa (NCTC 2000 and NCTC 6749) did not give a positive reaction with the indicator paper, nor did the strains of Serratia, nor strains GR, TE, and TI.

Recently Dr. F. Bustinza of the Academy of Sciences, Madrid, told me that the alkaline picrate test is also suitable. Filter paper is dipped in saturated aqueous picric acid, dried, dipped in 10% aqueous sodium carbonate, and dried again. A strip is placed between the plug and the side of a tube of a broth culture. If HCN is produced the yellow paper becomes brick red. Since the indicator paper is stable the cultures can be incubated for many days and the effective sensitivity of the method is thus very high. I observed that all mesophils (19 strains) gave positive results within 3 days at 25° with the exception of strain MH which gave a doubtful result. There was a little variation from strain to strain in the time required to give a positive result. None of the 24 psychrophils showed a positive reaction in 5 days.

Two strains of Serratia, and strains GR, TE, and TI gave negative results after 4 days. However, strong positive reactions were obtained with a strain of Pseudomonas chlororaphis (NRRL B.560) and 2 strains of Pseudomonas aureofaciens Kluyver, 1956 (NRRL B.1576 and 1543P) which had been provided by Dr. W. C. Haynes of Northern Utilization Research Branch, Peoria, Illinois, U.S.A. The picrate test thus seems more suitable for routine testing than the benzidine-copper test, but it should be remembered that the picrate test is not highly specific for cyanide (Anonymous, 1938). It may be more sensitive than the benzidine

copper test, since Pseudomonas aeruginosa strains NCTC 2000 and 6749 gave weakly positive reactions after 4 days.

Tryptophan metabolism. Mitoma, Weissbach and Udenfriend (1955, 1956) found that the strain of Chromobacterium here called RT converted L-tryptophan to L-5-hydroxytryptophan. This is probably the source of the 5-hydroxyindolyl residue in the pigment violacein. There was no evidence of further conversion to serotonin (5-hydroxytryptamine). Mitoma and his colleagues found very slight hydroxylation of D-tryptophan. Some 5-hydroxyindolylpyruvic acid was also produced, but none of the metabolites of the kynurenine pathway. Other indolyl compounds were not hydroxylated.

CHAPTER XIV

ANTIGENIC STRUCTURE

The first work on the serology of the genus appears to be that of Bampton (1913). He used flagellated suspensions and 'OH' antisera and found two main serological groups. Within each group the strains showed extensive cross-agglutination, but there was little cross-agglutination between the two groups. These groups corresponded to Bacillus violaceus (No. 108 in Appendix II) and B. membranaceus amethystinus (No. 102) and they were apparently all psychrophilic strains. In the former group some indistinct subgroups were found. Bampton's tables show evidence of a mixture of polar and peritrichous organisms in the agglutinable suspensions, since partial agglutination over a wide range of serum dilutions was found. Bampton found that precipitin and complement fixation reactions were more strain-specific than agglutination tests.

Soule (1939) and Dodd (1941, p. 27) found that their antisera were strain-specific, but Lesslar (1927) and Joubert and Nguyen-Van-Liem (1957) observed much cross-agglutination between strains isolated from infections and those from water, and this has been my own experience and that of Sippel (1955, pp. 52-62). Sneath et al. (1953) found that two pathogenic mesophilic strains (strains BH and BN) were almost identical antigenically, and that they cross-agglutinated with mesophils from water: they found that rough strains were agglutinated by many of the antisera and concluded that most mesophils possess common rough antigens. Sippel noted that whole organisms yielded better antisera than did extracts, and he found precipitin reactions were difficult to do.

Ramchandani (1930) and Kelner (1947) found no antigenic difference between parental strains and nonpigmented and lithium-resistant variants. Dodd (1941, p. 26) concluded that the bacteria were too anticomplementary to allow of reliable complement fixation tests. Davies (1955) showed that Chromobacterium possesses Boivin antigens—polysaccharide-lipid-protein complexes which are extractable with trichloracetic acid—which can be used in haemagglutination reactions (Davies, Crumpton, Macpherson and Hutchinson, 1958).

Much of the difficulty of elucidating antigenic structure was removed when it became apparent that strains possess two distinct flagellar antigens corresponding to the polar and lateral flagella, and that young agar

cultures possess both, but broth cultures possess only polar flagella. This has been discussed in detail in Chapter V under "Flagella." The lateral flagella give the rapid floccular agglutination characteristic of peritrichous bacteria like Salmonella, while the polar flagella cause a fine granular agglutination like O agglutination, but only after incubation overnight at high temperatures (as was noted in Vibrio by Gardner and Venkatraman, 1935). Polar flagellated suspensions seem to differ also in that formalin does not inhibit O agglutination (as it does when formolized peritrichous suspensions are titrated with anti-O-sera). This was noted by Gardner and Venkatraman (1935), Mayr-Harting (1948) and van den Ende (1952) with the polar flagellated Vibrio and Pseudomonas species. I have observed this with polar suspensions of Chromobacterium. Presumably the formalin makes the flagella rigid and with peritrichous bacteria this interferes mechanically with the contact between cell surfaces which is needed for O agglutination: with polar suspensions cell contact could still readily occur.

The somatic antigens behave in much the same way as those in other Gram-negative bacteria. There are evidently "smooth" O antigens and "rough" R antigens similar to those in Salmonella and Vibrio (White, 1935a, 1935b; Lyles and Gardner, 1958). The pattern of cross-reactions in Chromobacterium is similar to that in Pseudomonas, with a large number of ill-defined groups (Munoz, Scherago and Weaver, 1949; van den Ende, 1952), but, as described below, there are few cross-reactions between the mesophilic and psychrophilic groups.

RESULTS

The serology was done in collaboration with Dr. F. E. Buckland, and the data have been published (Sneath and Buckland, 1959). The technical details are given in Protocol 17 in Appendix I. Most of the data relate to somatic antigens. The study of H antigens has been restricted to the investigation of the unusual two types of flagella (see Chapter V) and to some of the strains isolated from infections.

Antisera. Antisera were produced in rabbits using "boiled" organisms for 'O' antisera and formolized organisms for 'OH' antisera.

Effect of various treatments upon the suspensions used for agglutination. As shown in Chapter V broth cultures possess only polar flagella while young agar cultures possess both polar and lateral flagella, and the two forms of flagella are antigenically different. Formolization of polar suspensions does not inhibit O agglutination: formolization of peritrichous suspensions usually inhibits somewhat (Protocol 17). Some experiments with boiled and alcohol-treated suspensions are also shown in Protocol 17. Both of these treatments tend to decrease the titre with the homologous O antiserum and to raise the titre with O antisera to other strains. Such suspensions were noted to become more autoagglutinable in physiological saline, i.e. they became artificially "rough." For these reasons living suspensions were used for study of somatic antigens. The culturally rough strains were autoagglutinable in physiological saline, but by making the titrations in one-fifth strength saline (0.17% NaCl) it was possible to work with these strains as there was then very little agglutination in the control tubes.

Somatic antigens. In the early part of the work antisera were made to all the strains, but when a large number of psychrophils were received it was not practicable to do this, and only a few antisera against psychrophils were prepated. The large number of possible combinations of suspension and antiserum made it necessary to test these first by slide agglutination (using living suspensions from agar cultures and undiluted antiserum), in order to economize on antiserum. Only those showing agglutination were then repeated by titration in tubes. This screening procedure appears reliable since O, polar H, and lateral H agglutination are all readily seen by slide agglutination.

The results of somatic agglutination are shown in Tables 8 and 9, (using living broth cultures and 'O' antisera). Most of the reactions are probably due to O antigens, though with the rougher strains they may be partly due to R antigens.

It is seen from Table 8 that the mesophilic strains show considerable cross-agglutination to high titre. This is most marked with rough strains, while the agglutination of smooth strains is more strain-specific. This suggests that many rough strains possess R antigens in common, and probably there are some R-antibodies in the sera made by injecting smooth strains. The behaviour of two partially rough variants BHR/1 and BNR/1 (obtained by growing strains BH and BN, respectively, in broth containing anti-O serum, and plating out the cultures) supports this. These rough variants are agglutinated by antisera which do not agglutinate the parent strains and BHR/1 has lost the O antigens responsible for the agglutination of BH and BN by the homologous sera. The antisera to the rough variants agglutinate the smooth parental strains very poorly. Since these rough variants were autoagglutinable in saline, gave "rough" colonies and were poorly virulent (see page 327) the parallel with the S-R variation found in other organisms is very close.

There are no clear-cut serological subgroups within the mesophilic group (C. violaceum), though several strains are very similar antigenically such as the pairs BH and BN and FH and LG. The atypical mesophils RT, MH, SH, and LW which Leifson (1956b) regards as a distinct species do not form a distinct serological subgroup, and, in general, there seems little correspondence between the serology and other characters of the strains.

In Table 9 are shown the results of agglutination of psychrophilic strains with antisera prepared against several psychrophils. All these strains were culturally smooth, and here again the reactions are mainly strain-specific. A few cross-agglutinations are seen, which suggests that the position in Chromobacterium lividum is probably similar to that in the mesophils, with many rather indistinct serological subgroups.

Cross-agglutination between mesophils (C. violaceum) and psychrophils (C. lividum). The great majority of the tests of antisera against mesophils upon psychrophilic strains (and vice versa) were negative. Out of 460 such combinations of 'O' antisera and strains, only ten showed positive agglutinations in tube tests. They are, therefore, not given in tables but are listed below:

Table 8. Titres of O agglutination with mesophilic strains of antisera prepared against mesophils. 'O' antisera *versus* living broth cultures incubated 4 hours at 56° and then 18 hours at room temperature.

Suspensions of strains:	Antisera against strains:															
	SL	BN	BH	BNR/1	BHR/1	LG	FH	RT	MH	AM	SH	LW	MW	TV	DK	MK
SL(S)	640	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
BN(S)	80	160	320	20	40	0	0	0	20	0	0	0	40	160	160	320
BH(S)	0	160	320	20	0	0	0	0	20	0	0	0	0	0	0	0
BNR/1(r)	-	160	160	640	320	320	320	0	-	0	0	320	320	320	-	320
BHR/1(r)	-	0	0	0	80	320	320	0	-	0	0	0	0	0	-	0
LG(S)	0	0	0	0	40	1280	160	0	80	0	0	0	0	0	0	0
FH(S)	0	320	20	160	320	320	5000	320	160	0	0	0	0	0	0	0
RT(R)	0	0	80	0	40	320	80	320	320	0	0	0	0	160	0	80
MH(S)	0	0	0	0	0	0	0	40	640	160	80	80	40	20	0	10
AM(R)	0	0	40	40	80	0	160	0	80	1280	0	80	0	160	160	320
SH(R)	0	0	0	0	0	160	320	0	160	0	160	0	0	0	20	0
LW(R)	0	0	20	40	20	0	320	320	320	0	640	640	0	80	0	80
MW(R)	0	0	0	0	0	0	20	0	160	160	40	0	640	320	20	40

TV(S)	0	0	40	0	0	0	0	0	0	0	0	0	160	640	0	40
DK(S)	0	0	80	0	40	0	0	0	0	0	0	0	0	0	320	80
MK(S)	0	0	0	0	0	0	0	0	0	0	0	320	0	0	160	1280
RV(r)	0	20	0	0	0	20	0	20	0	40	0	20	10	40	160	40
TA(S)	0	0	0	0	0	0	0	0	0	0	0	0	40	40	0	20
TB(S)	0	0	0	0	0	0	0	0	0	0	0	0	0	10	0	0
PT(S)	0	10	20	20	0	0	0	0	0	0	0	0	0	0	0	40

0 = less than 10. - = not tested.
The approximate degree of 'roughness' of the strains is indicated by the symbol in brackets after the strain letters:

(R) = markedly rough, giving considerable autoagglutination in 0.9% NaCl;
(r) = slightly rough, giving only slight autoagglutination;
(S) = smooth, giving no autoagglutination.

The strains from cases of natural infections and their sources are:

SL, Sealy strain from pig (Sippel et al., 1954); BH and BN from man (cases 11 and 12 in Sneath et al. 1953; SH, Shahan strain from man (Black and Shahan, 1938); DK, from man (Darrasse et al., 1955); RV, Reeves strain from cattle (Sippel et al., 1954) and PT, Porter strain from pig (Sippel, 1955): see pp. 343, 344.

Table 9. Titres of O agglutination with psychrophilic strains of antisera prepared against psychrophils. 'O' antisera *versus* living broth cultures incubated 4 hours at 56° and then 18 hours at room temperature.

Suspensions of strains:	Antisera against strains: NT	EC	NC	PB	HB	HE	MB
NT	160	0	0	0	0	0	0
EC	0	160	40	0	0	0	0
NC	0	0	320	160	0	0	0
PB	0	0	10	160	0	0	0
HB	0	0	0	0	2560	0	0
HE	0	0	0	0	40	80	0
MB	0	0	0	0	0	0	160
EA	0	80	0	0	0	0	0
HD	0	0	160	80	0	0	0
GA	0	0	80	0	0	0	0
MA	0	0	160	0	0	0	0
DA	0	0	0	40	0	0	0
IN	0	0	0	20	10	0	0
HA	0	0	0	0	40	10	0
EB	0	0	0	0	0	0	0
MC	0	0	0	0	0	0	0
HC	0	0	0	0	0	0	0
HF	0	0	0	0	0	0	0
CA	0	0	0	0	0	0	0
RU	0	0	0	0	0	0	0

0 = less than 10.
All these psychrophilic strains were culturally 'smooth.'

1. Agglutination of mesophils by antisera against psychrophils: anti-NT had a titre of 160 with strain AM and of 40 with strain LW; anti-HE had a titre of 40 with strain AM and of 80 with strain MH; anti-EC had a titre of 20 with strains MH and DK; anti-MB had a titre of 80 with strain MH; anti-HB had a titre of 160 with strain MH.

2. Agglutination of psychrophilic strains by antisera against mesophils: anti-LG had a titre of 320 with strain NT; anti-MK had a titre of 20 with strain NT.

Probably most of these cross-reactions are not due to common antigens, since normal rabbit sera may agglutinate strains of Chromobacterium to a titre of 20 or 40.

Antigenic relationship of strains of Chromobacterium violaceum causing natural cases of infection. Strains causing infections were not found to form a homogeneous serological group. This can be seen for O antigens from Table 8, where, for example, there is no cross-agglutination between strains SL and DK. There may perhaps be rather more cross-agglutination between the pathogenic strains SL, BH, BN, and PT than would be expected, but this is probably not significant. The H antigens of five strains were examined in more detail, and the results are shown in Table 10. It was not possible to prepare pure polar H antisera (see Chapter V) so that the titres in Section I represent mixed O and polar H agglutination. The 'OH' antisera, although prepared by injection of polar suspensions, contained enough lateral H antibody for the experiment. It is seen that there is no H antigen common to all the strains, but strains SL, BN and presumably also BH have a lateral H antigen in common. There is some suggestion that SH possesses a polar antigen in common with BH and BN, since these cross-agglutinations in Table 10, Section I, do not seem to be due to somatic antigens (see Table 8).

Antigenic relationship of Chromobacterium to other bacteria. All the antisera against strains of Chromobacterium were tested by slide-agglutination against living suspensions of two strains of Whitmore's bacillus, against "Chromobacterium iodinum" (strains TE and RE) against "Chromobacterium viscosum" (strain GR), against two strains of Pseudomonas aeruginosa (NCTC 2000 and NCTC 6749) and against six stock strains of Serratia marcescens. No agglutination was found in any instance. Several mesophils were similarly tested with a range of diagnostic antisera to Salmonella, Shigella, Brucella, Whitmore's bacillus and the cholera vibrio, and no agglutinations were observed. Sippel (1955, p. 64) obtained negative results with a similar range of antisera. Strain TI ("Chromobacterium ianthinum" of Gilman, see No. 59) was tested with all the Chromobacterium antisera, and all strains of Chromobacterium were tested with an 'OH' antiserum against strain TI; a few doubtful reactions were seen which were negative when tested in tubes. The negative findings are in keeping with the opinion that the other chromogenic bacteria mentioned above do not belong to the genus Chromobacterium (Sneath, 1956a), and no serological relationship to other genera has yet been found.

Table 10. Agglutination due to flagellar antigens.

I. Agglutination due to O and polar H antigens. Agglutination titres of antisera containing O and H antibodies with living suspensions possessing polar flagella but no lateral (peritrichous) flagella, incubated at 56° for 18 hours.

Suspensions of strains:	Antisera against strains: BH	BN	SL	SH	DK
BH	80	160	160	80	160
BN	1280	320	640	160	40
SL	10	80	640	0	0
SH	80	40	20	640	10
DK	40	160	80	40	640

II. Agglutination due to lateral (peritrichous) H antigens. Agglutination titres of H antisera (absorbed with homologous boiled organisms to remove O antibodies) with suspensions possessing lateral (peritrichous) flagella, incubated at 56° for only 2 hours to avoid agglutination due to polar flagella.

Suspensions of strains:	Antisera against strains: BH	BN	SL	SH	DK
BH	no peritrichous suspensions obtainable				
BN	1280	160	160	0	0
SL	640	40	80	10	0
SH	0	0	0	320	0
DK	0	0	0	0	1280

0 = less than 10.

CHAPTER XV

CHEMICAL STRUCTURE

Carbohydrates. Davies and his colleagues have examined the composition of the polysaccharides associated with the material extractable with trichloracetic acid (Boivin-type lipopolysaccharide substances, presumably mainly O antigen and perhaps also cell-wall components). The carbohydrates found in a study of eleven strains (ten mesophils and one psychrophil (strain NT)) are shown in Table 11, taken from the papers by Davies (1953, 1955, 1957), Crumpton and Davies (1956, 1958), and Maclennan and Davies (1956, 1957) and, for strains DK, SL, RV, personal communications from Davies. Crumpton and Davies (1958) noted that in strain NT the glucosamine appears to be attached to the lipoid moiety of the lipopolysaccharide, not to the polysaccharide moiety.

Table 11. Carbohydrates in the polysaccharides of Chromobacterium.

	Strain										
Carbohydrate	BH	BN	LW	MK	AM	MW	FH	DK	SL	RV	NT
Glucose	+	+	+	+	+	+	+	+	+	+	+
Rhamnose	+	+	-	+	-	+	+	+	+	+	-
Galactose	-	-	+	-	-	+	-	tr	+	tr	+
Xylose	-	-	+	-	-	+	-	tr	-	tr	-
Arabinose	-	-	+	-	-	-	-	-	-	-	-
Fucose	-	-	tr	+	-	-	-	+	+	-	-
Mannose	-	-	-	-	-	-	-	+	-	tr	-
D-manno-D-galaheptose (a)	+	+	-	-	-	-	-	-	-	-	-
D-glycero-D-mannoheptose (b)	-	-	-	-	-	-	-	-	-	-	+
Galacturonic acid	-	-	+	-	-	-	-	-	-	-	-
Glucosamine	+	+	-	-	-	-	tr	+	+	+	+
Galactosamine	-	-	-	-	-	-	-	-	tr	+	-
Unknown hexosamine	-	-	-	-	-	-	+	+	-	-	-
D-fucosamine (c)	-	-	-	-	-	-	-	-	-	-	+

+ = present in moderate or large amounts; tr = trace;

(a) Also called D-glycero-D-galactoheptose (Maclennan and Davies, 1956, 1957);

(b) Crumpton and Davies (1956), Davies (1957);

(c) Probably N-acetylated (Crumpton and Davies, 1958).

In Salmonella Davies (1955) observed close correlation between the O antigens and the carbohydrates in the polysaccharide. There is no very clear correlation in Chromobacterium though BH and BN have identical sugars and are also antigenically very similar (Table 8, p. 316).

Miscellaneous. Glutathione occurs in Chromobacterium as in most bacteria (Miller and Stone, 1938). Anderson et al. (1958) found the contents of lysine, methionine, and tryptophan in Chromobacterium to be very close to the figures found for other Gram-negative bacteria; they comprised approximately 8%, 2%, and 0.2% of the protein, respectively. Both chitin and cellulose are absent from Chromobacterium (as from all bacteria examined) according to van Wisselingh (1898). Fatty material was found to be abundant in the cells by Burdon (1946); as mentioned in Chapter V this seems typical of mesophilic strains. Forsyth, Hayward, and Roberts (1958) found that over 30% of the dry weight of two mesophilic strains grown on glucose peptone medium consisted of a polymer of β-hydroxybutyric acid. This substance (M.Pt. 164-168°) was soluble in chloroform, but not in ether, and is probably responsible for the staining of mesophils with fat stains. It was also noted in Rhizobium and some pseudomonads, especially the group to which strain TI (No. 59) belongs (Morris and Roberts, 1959; Hayward et al. 1959). The gelatinous material produced by the membrane-forming psychrophilic strains is probably composed of carbohydrates: it is soluble in boiling water, in dilute alkalies (0.1 N NaOH) or in strong acids (N HCl) according to Corpe (1953). However, after boiling with hydrochloric acid it did not reduce Fehling's solution. Corpe (1958) found that it forms about 15% of the dry weight of gelatinous strains. It surrounds the cells and shows a fibrillar structure in electron micrographs, and does not readily leach off the cells. He extracted it with alkali and purified it. It gave a viscous solution in water, and was composed of glucose, a methyl pentose and a hexosamine in the ratio 7:1:1, and also contained as an integral part two amino-acids, one of which was aspartic acid. Fuchs (1959, p. 33) reported that Chromobacterium did not synthesize levan. O'Connor, McCall and DuPré (1957) published the infrared absorption spectrum of whole cells of an unnamed species of Chromobacterium. It was similar to those of Pseudomonas fluorescens and Bacillus polymyxa, but acetone extracts of the three organisms gave very different spectra.

CHAPTER XVI

VARIATION AND BACTERIOPHAGE

Variation

Variation in pigmentation. The most striking variation in Chromobacterium is the whole or partial loss of pigment, which has been noticed by most workers. This is common on continued subculture, and probably also occurs in infected animals (Gauducheau, 1907). Eisenberg (1914) observed several variants, some colourless, some pale violet, which bred true, but threw off occasional further variants. Ramchandani (1930) studied the problem and observed that even the palest colonies produced

some pigment after long incubation. On continued subculture, few deeply pigmented colonies were found, though be observed reversion to a more deeply pigmented form occasionally. He noted fewer variants on medium of acid pH. Waeldele (1938, pp. 73, 91) found that media containing glucose and oligosaccharides encouraged the appearance of nonpigmented variants. He observed that pigmentation was often regained by passage on starch, dextrin, or glycogen media, or potato. Media containing glycerol, glucose, and other simple sugars were not effective. Apparently Macé (1888b) was the first to observe that passage on potato would restore the pigment-forming ability, and Dmitrevskaia (1936), Dodd (1941, p. 21) and Morris (1954) have also reported this. Morris also used oatmeal agar with success. Dmitrevskaia found that the addition of 0.03% of potassium nitrate or nitrite to peptone media was effective. Morris noted some effect on adding 0.5% of lithium chloride to the media.

My own observations have not been very extensive. Both mesophilic and psychrophilic strains commonly throw off pale violet or white variants, and generally there are several degrees of pigmentation. A puzzling observation is that a strain after remaining stable for several subcultures will suddenly throw off these variants and persist in this for several more subcultures, and then gradually or suddenly lose the tendency to do so. White colonies sometimes become pale yellow on longer incubation as noted by Martin (1931). Occasionally two pale variants of one strain are seen, one forming most pigment in the centre of the colony and the other at the edge. I have found the easiest way in which to obtain pigmented subcultures from cultures which have lost almost all their pigment is to make a heavy inoculum on agar and observe the edge of the confluent growth: narrow pigmented sectors are usually seen, which can be picked with a fine needle and subcultured. This succeeds when no violet colonies can be found upon direct plating, and has, I believe, been used for this purpose in Serratia.

Variations in colonial form. Eisenberg (1914) observed dissociation into flat and convex colonies which bred true. Ramchandani (1930) described smooth, matt and glossy colonies and Floch and de Lajudie (1943) noted colonies which were flat and rugose with an irregular edge. The secondary papillae and sectors which develop in old cultures have been studied extensively (Hutchison, 1940; Hutchison and Kelner, 1942; Kelner, 1947). They are common in cultures containing high concentrations of salts. On agar containing lithium chloride (0.12 Molar) the papillae soon overgrew the colony, and they are composed of cells with a high resistance to lithium salts. The toxicity of lithium chloride depends on the pH, being greater at alkaline pH, and since the medium is made alkaline by growth, the wild type cells become inhibited. The lithium-resistant variants will revert to the parental form on subculture several times in plain broth. With other alkali metals (sodium chloride 0.2M, potassium chloride 0.25M, rubidium chloride 0.2M and caesium chloride 0.05M) various other forms of papilla were seen. Hutchison (1940) also described four colonial variants on ordinary media, the parental smooth S form, composed of single organisms, the R form with rough colonies composed of chains of organisms which were autoagglutinable in saline, and two intermediate types. The SR form was colonially smooth but consisted of short chains of organisms and the RS form was colonially rough, but was

composed of single organisms. My own experience has been similar to that of Hutchison. The normal form is quite smooth, but variants of different degrees of roughness and of autoagglutinability soon appear on subculture, and it is difficult to maintain the parental form for work on virulence and serology.

Variations in pathogenicity. These are discussed later, but as in most bacteria, rough variants are poorly virulent.

Variations in the gelatinous (membranous) habit. Corpe (1953) described the dissociation of the membrane-forming gelatinous strains into nongelatinous variants. My experience has been the same. Deshusses and Novel (1939) seem to have observed a similar phenomenon.

Other variations. Ramchandani (1930) noticed variants with a slightly different optimum temperature, with different growth rates and also variants which fermented dextrin. Floch and de Lajudie (1943) noted variants which differed in their haemolytic action. Deshusses and Novel (1939) found that colonies varied in their power to liquefy gelatin, and Lehmann and Neumann (1899, Vol. 2, p. 262) found that this ability was often lost on continued subculture. I have noted streptomycin resistant mutants, loss of ability to reduce nitrates, and a variant which fermented starch promptly.

Sexuality. There are no records of any recombination, transformation, or transduction in Chromobacterium. The evidence of Enderlein (1925, pp. 150, 154-6, 177) and of Kisitani and Sumiyosi (1939) for a life-cycle involving gonidia and other such entities is quite valueless.

Bacteriophage

Sertic and Boulgakov (1935) reported the isolation from sewage of bacteriophages active against Chromobacterium. They found them to have a low resistance to heat, being killed within 1 hour at 52°. Their five phages differed in the size of the plaques, in the degree of lysis they gave and in the ease with which phage-resistant variants were found. None of these phages attacked any of a selection of coliform and dysentery bacilli, Pseudomonas, Staphylococcus, or Serratia. Rhizobium phages do not attack strains of Chromobacterium or Agrobacterium (Bruch and Allen, 1957; Parker and Allen, 1957).

I have tested twenty-seven strains of Chromobacterium with a wide range of bacteriophages kindly supplied by Dr. I. N. Ascheshov (see Protocol 18 in Appendix I). None of the mixtures lysed any strain. As with serology a negative finding is of little significance in classification.

CHAPTER XVII

PATHOGENICITY

Mesophilic strains of Chromobacterium (C. violaceum) are sometimes the cause of severe infections in man and animals; they are discussed fully in Chapter XIX. There is a good deal of information upon the pathogenicity as studied in experimental animals, but some of the early workers do not make it clear whether they were using psychrophilic strains (C. lividum) which, since they do not grow at 37°, would not be virulent for warm-blooded animals. Thiry (1900, p.108) records a possible case of infection in a plant (Tricholoma saponaceum). Roncali (1893) injected Chromobacterium together with Clostridium tetani into guinea pigs and the animals died of tetanus. Massa (1889, quoted by Bertarelli, 1903a, 1903b) found Chromobacterium to be weakly pathogenic if mixed with Serratia marcescens, but Bartarelli himself found that only the latter organism could be cultured from guinea pigs which died. Chromobacterium alone was only lethal in huge doses. Bréville (1897, p.51) reported that Chromobacterium increased the virulence of Pseudomonas aeruginosa in animals. Ward (1897, 1898) found that psychrophils were not virulent for animals, as did Fuller and Johnson (1899). Bampton (1913) tested one psychrophilic strain and found it nonvirulent and nontoxic on injection into mice, guinea pigs, dogs and rats.

Most of these reports did not distinguish clearly between toxicity of the injected material and multiplication of the organisms in the host, which may be conveniently distinguished under the headings of toxicity and virulence.

Virulence

So far as is known virulence is only found among mesophilic strains, i.e. Chromobacterium violaceum.

Susceptible animals. Natural infections have occurred in man, buffaloes, swine, cattle, and monkeys (see Chapter XIX). In experimental animals some strains are virulent for monkeys (Woolley, 1904, 1905; Audebaud et al., 1954; Joubert and Nguyen-Van-Liem, 1957), for dogs (Woolley, 1904; 1905), for cats (Woolley, 1904, 1905; Christopher and McCleskey, 1941) for swine (Sippel et al., 1954) and for calves (Woolley, 1904, 1905). Most of these authors and several other workers have found guinea pigs, rabbits, rats, and white mice to be susceptible.

There is little information on which animals are the most susceptible. The work of Woolley suggests that dogs, cats, and cattle are relatively resistant, and the work of Schattenberg and Brown (1941) and Sippel (1955, pp.14-34) suggests that mice are more susceptible than guinea pigs or rabbits. It seems likely from the data of these authors that the LD_{50} for swine, guinea pigs and rabbits was 10^8 to 10^9 viable organisms by injection. The strain BN is a good deal more virulent than this (see below).

Virulence on injection. There is general agreement with Woolley (1904, 1905) that large doses cause death from septicaemia within a day

or two, while small doses commonly produce multiple abscesses in various organs, with death after several days or weeks. Small doses sometimes produce only a local abscess.

Virulence by mouth. Most authors have found that mesophilic strains which were virulent on injection were not virulent when given by mouth to monkeys (Woolley, 1904, 1905), to guinea pigs (Minett, 1913), to guinea pigs, mice or rabbits (Sneath et al., 1953). Hanssen (1912) fed large amounts of a strain of Chromobacterium to a dog without ill effects. and the organism did not establish itself in the bowel. However, Sippel (1955, pp.15-18) was able to infect pigs by mouth, and believed that this was the route of infection in the epidemic he observed in pigs.

Other observations. Woolley (1904, 1905) was unable to infect a dog by intratracheal injection of a virulent strain. Sippel (1955, p.49) noted that a strain would kill 10-day chick embryos on chorio-allantoic injection. Joubert and Nguyen-Van-Liem (1957) were able to produce pneumonia in mice, rabbits, and guinea pigs by intranasal inoculation.

Virulence of strains from cases of natural infection. Woolley (1904, 1905) first showed that strains from infections were virulent for experimental animals, and could be passaged in guinea pigs. Soule (1939) and Dodd (1941, p.30) confirmed the virulence of the strain of Black and Shahan (1938), which is strain SH, but which is now rough and avirulent. Soule's own strain was poorly virulent. Sippel (1955, pp.14-34) conformed the virulence of strains BH and BN. He found strains SH and RT to be avirulent, and strain RV to be virulent. Audebaud et al. (1954), Darrasse et al. (1955), Floch and de Lajudie (1943), Lesslar (1927), Schattenberg and his colleagues (Schattenberg, 1940; Schattenberg and Brown, 1941; Schattenberg and Harris, 1941, 1942), Sneath et al. (1953) and Joubert and Nguyen-Van-Liem (1957) all reported that their strains were virulent. The strains of Martin (1931) and of Sartory et al. (1938a) 1938b) were avirulent for guinea pigs, but the latter was probably not from a true infection (Waeldele, 1938, p.55).

Virulence of strains isolated from water. Many authors have isolated mesophilic strains of Chromobacterium from water which were virulent on injection into laboratory animals (Gauducheau, 1907; Minett, 1913; Lesslar, 1927; Broudin, 1922; Sneath et al. 1953; Sippel, 1955, pp.14-34). This is not surprising, since strains causing infections are presumably derived from water and similar sources, and Sippel gives evidence that an outbreak in pigs was derived from the drinking water of the animals. Woolley (1904, 1905) and Schattenberg and Harris (1942) imply that virulent strains may be a distinct variety, but this seems unlikely since I have been unable to find any characteristic, either cultural or antigenic, which is correlated with virulence.

Loss of virulence on subculture. Some workers have noted a fall in virulence on subculture (Gauducheau, 1907; Sippel, 1955, p.14; personal observations). Schattenberg and Harris (1942) however, found that some strains could retain their virulence for many months of continuous subculture.

Experiments on virulence. In conjunction with Dr. F.E. Buckland (Sneath and Buckland, 1959) extensive tests have been made with most of the mesophilic strains and one psychrophil (NT), and the results may be summarized as follows.

Several strains are virulent for white mice on intraperitoneal injection, the LD_{50} of living organisms being about 10^6 for the most virulent strains. The organism caused septicaemic death, as a rule within 24 hours. Occasionally a mouse lived for a week or two, and showed wasting, diarrhoea, and on post-mortem small abscesses in the liver and spleen. The organism was easily isolated from these abscesses.

In guinea pigs fewer strains showed high virulence, and for the most virulent strains (BN and BH) the LD_{50} of living organisms was between 10^6 and 10^7. Again the usual mode of death was by septicaemia within 24 hours, but occasional animals after a week or two might show hepatic and splenic abscesses from which the organism could be generally isolated. Strains BH and BN were highly virulent for rabbits on intravenous injection: Mr. G.J. Harper injected 1 ml of an overnight broth culture on each strain into a number of rabbits, and found that most of the animals died of septicaemia within 48 hours. A few survived for several days and at autopsy they showed abscesses in the liver, lungs, and spleen. The LD_{50} was not determined.

Nine strains were passaged through guinea pigs and the virulence determined before and after passage. There was definite but slight increase in virulence (as shown by the LD_{50}) with a few strains. Dodd (1941, pp. 46-48) was also unable to increase the virulence of strains to any great extent by passage.

These results are summarized in Table 12 below and the details are given in Protocol 19 in Appendix I. The post-mortem appearances are discussed in Chapter XX (p. 348).

It is clear from Table 12 that many strains, including the psychrophil NT, cause death mainly by the toxicity of the inoculum, since huge doses were required. The rough variants of BH and BN (strains BHR/1 and BNR/1, see Table 2, p. 262) are much less virulent than the parental strains. Dr. F. E. Buckland kindly determined the approximate LD_{50} of some of these avirulent strains by injecting massive doses of living organisms into guinea pigs intraperitoneally. He found the following LD_{50}: strain AM, 2×10^{10} organisms; strain MW, 5×10^8 organisms; strain TV, 1×10^{10} organisms; strain NT, 2×10^{10} organisms; strain LW, 2×10^{10} organisms.

Mucin is well-known to enhance the virulence ot many bacteria. Mr. G.J. Harper, using strain BH, tested the effect of mucin by estimating the LD_{50} in mice. The inoculum was a nutrient broth culture grown at 37° for 18 hours, from which ten-fold dilutions were made in parallel in broth and in broth containing 1.75% of mucin (batch 1701 W of granular mucin of Wilson's Laboratories, Chicago). 0.5 ml quantities of the dilutions were injected intraperitoneally into batches of ten mice, and the results are shown in Table 13.

It is seen that the LD_{50} was reduced by mucin from about 2×10^6 organisms to about 8×10^2 organisms.

Dodd (1941, pp. 33, 57, 65-69) reported that cultures grown in a mixture of 25% carbon dioxide in air were more virulent and contained more of a toxic filtrable factor than cultures grown in air alone. Dr. F.E. Buckland investigated this and has provided the following data. Cultures of BN, SH, and LG were grown on semi-solid nutrient agar containing 5% of peptic digest of sheep blood, and incubated at 37° for 18 hours in

Table 12. Virulence of Chromobacterium strains for mice and guinea pigs.

The organisms were grown in nutrient broth cultures for 18 hours at 37° except for strain NT which was grown at 25° for 48 hours. Viable counts were made, and after making suitable dilutions each dilution was inoculated intraperitoneally into ten white mice or five guinea pigs. The approximate LD_{50} was obtained by interpolation. Some strains were passaged five times in guinea pigs and re-tested.

	Approximate LD_{50} of living organisms for		
		Guinea pigs	
Strain	Mice	before passage	after passage
FH	over 10^8	10^9	10^8
MK	10^6	10^8	10^8
BH	2×10^6	10^7	10^6
BHR/1	10^7	5×10^9	5×10^8
BN	10^6	5×10^6	10^6
BNR/1	10^7	10^9	5×10^8
AM	10^8	2×10^{10}	-
MW	over 10^8	5×10^8	-
TV	5×10^7	10^{10}	-
LG	10^7	10^9	10^7
LW	over 10^7	2×10^{10}	-
SH	over 10^7	10^9	10^8
RT	over 10^7	5×10^9	10^8
NT	over 10^7	2×10^{10}	-

- = not tested.

25% CO_2 plus 75% air. Control cultures were incubated in air. The growth was washed off with potassium phosphate buffer (0.04 M, pH 7.4). Part was Seitz-filtered. Suitable dilutions were injected intraperitoneally into guinea pigs and viable counts were performed on the suspensions. The approximate LD_{50} was determined using 5 animals for each dilution) and are shown below:

Strain	Approximate LD_{50} of viable organisms Grown in air	Grown in 25% CO_2
BN	5×10^5	5×10^6
SH	2×10^9	1×10^9
LG	5×10^8	5×10^8

Table 13. The effect of mucin upon virulence.

Batches of 10 mice were inoculated intraperitoneally with 0.5 ml of living suspensions of strain BH suspended in broth or in broth containing 1.75% of mucin.

Number of viable organisms injected	Number of deaths within 7 days	
	Without mucin	With mucin
8.0×10^6	10	-
8.0×10^5	0	10
8.0×10^4	0	9
8.0×10^3	0	9
8.0×10^2	0	5
8.0×10^1	-	1
0	0	1

- = not tested.
Approximate LD_{50} without mucin = 2×10^6 organisms.
Approximate LD_{50} with mucin = 8×10^2 organisms.

The filtrates were nontoxic when 1 ml was injected intraperitoneally into guinea pigs. The findings of Dodd were not confirmed, as the differences between the cultures grown in air and 25% CO_2 are not significant. However, the strains were different from Dodd's (except for SH, which has become avirulent), and there may also be other explanations (see below).

Toxicity

Endotoxins. There is no doubt that virulent mesophilic strains of Chromobacterium contain a toxic material within the cells. I have on several occasions killed rabbits by injecting too large an initial dose of boiled suspensions in preparing antisera. The animals died in a few hours, and boiled suspensions seem to be much more toxic than formolized suspensions of the same concentration of cells, though this point has not been carefully examined. Sippel (1955, pp. 29, 50) has also noted that formolized organisms were nontoxic. Woolley (1904, 1905) found that suspensions heated at 57° for 1 hour were nontoxic to monkeys and dogs on injection, and Minett (1913) found heat-killed broth cultures to be nontoxic to guinea pigs on injecting 1 ml. However, Darrasse et al. (1955) state that heat-killed organisms were very toxic to mice and guinea pigs, so it is likely that strains differ in their toxicity. Joubert and Nguyen-Van-Liem (1957) reported that trichloracetic acid extracts were nontoxic.

Exotoxins. Woolley (1904, 1905) found that filtrates of broth cultures were nontoxic to a monkey on injecting 5 ml. Minett (1913), Schattenberg (1940), and Darrasse et al. (1955) likewise found no evidence of an exotoxin, although Schattenberg observed that the filtrates had some power

of protective active immunization in mice. As mentioned above Dodd (1941, pp. 65-69) claimed that cultures grown in 20 to 25% carbon-dioxide air mixtures were more toxic than those grown in air. Intradermal injection into rabbits and guinea pigs produced large oedematous local lesions. The toxic factor was stable to heat (60° for 30 minutes) and to phenol (0.5% for 6 hours), both of which treatments killed the cells. Much of it was outside the cells, and culture filtrates were very toxic, since 1 ml of filtrate of a broth culture grown in 25% CO_2 would kill rabbits on intravenous injection. All Dodd's strains produced this toxin, though the most virulent was also the most toxic. The factor disappeared on storing at 4°, and was antigenic. The high lethality on intravenous injection suggests a haemolysin as the main component. Sippel (1955, pp. 29, 50) found that culture filtrates were toxic to rabbits but much less so to guinea pigs; on subcutaneous injection they produced only local lesions in guinea pigs and swine. The rabbits on post-mortem showed pronounced centrolobular fatty change in the liver. Joubert and Nguyen-Van-Liem (1957) and Nguyen-Van-Liem (1957) made similar observations to those of Sippel. As has been noted in Chapter VI most mesophilic strains of Chromobacterium are haemolytic on blood agar plates.

Experiments on toxicity. As noted above, Dr. Buckland was not able to confirm the findings of Dodd. The results given above show that Chromobacterium does not contain a potent endotoxin, though differences between strains, in the susceptibility of different animals and in the route of injection may play an important part, and this has not been systematically studied. Dr. Buckland and Mr. Harper examined strain MK in some detail. Heavy suspensions from nutrient agar slope cultures grown in air at 37° for 24 hours were prepared, containing about 2×10^{10} viable cells per ml. Part was heated at 100° for 30 minutes, part was Seitz-filtered and part was not treated. The three preparations were injected intraperitoneally into batches of five guinea pigs in 1 ml amounts Only that containing living cells caused death of the animals (all five animals died).

Dr. Buckland examined a few strains for the production of soluble haemolysin. In preliminary tests he found that the addition of 0.5% of glucose or 10% of horse serum to the broth used for growing the strains had little effect on haemolysin production. Nutrient broth cultures (tryptic meat digest) grown at 30° for 18 hours were mixed with equal volumes of washed 3% rabbit red-cells and incubated at 37° for 1 hour. The strains MK, BH, BN, BHR/1, AM, MW, SH, RT, and LG caused marked lysis. The strains FH, TV, and LW showed slight lysis. Sheep and horse-red-cells gave much less lysis than rabbit red-cells. Probably the lysis is due to lecithinase as these strains give a positive egg-yolk reaction (see p. 309) and presumably much of the rather low toxicity of culture filtrates is due to the lysins.

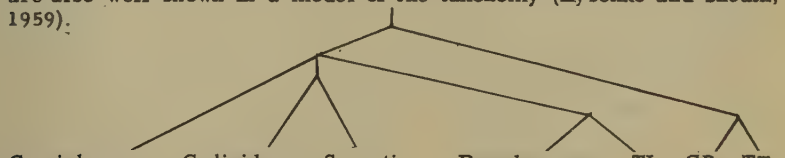
CHAPTER XVIII

THE CLASSIFICATION OF CHROMOBACTERIUM

The systematic position of the genus

At present the theory of bacterial taxonomy is going through a period of considerable change. It is being realized that species which are founded on a single strain (examined commonly by only a few tests) are very unsatisfactory, and that far more detailed work on many strains is necessary to produce good taxonomic groups. Elsewhere (Sneath, 1957a, 1957b) I have proposed a new approach to bacterial taxonomy. This rests on the theoretical basis that orthodox scientific taxonomy is concerned with the over-all similarity between creatures, taking into account all their features, that every feature should logically be given equal weight, and that divisions into taxa are based upon correlctions between features. A practical method of estimating over-all similarity was developed which employs an electronic computing machine to perform the laborious and mechanical procedure of comparing the strains.

This method has been tried upon the data which I have collected for Chromobacterium (Appendix to Sneath, 1957b) and it confirmed the validity of the separation into mesophilic and psychrophilic groups, for there was a pronounced gap between the values for mesophils and for psychrophils. Within the mesophilic group three rather indistinct subgroups were indicated: These strains were (1) MK, DK, TA, TB, TV, and LG; (2) BH and BN; (3) LW, RT, and SH. Within the psychrophilic group there were subgroups composed of (1) strains DA, NC, HD, MA, and MC; (2) HA, RU, CA, HB, and IN; (3) EA, EB, and EC. They do not seem to be sufficiently well marked taxonomic entities to require varietal names. The most aberrant mesophilic strains were FH and AM; the most aberrant psychrophils were NT, MB, and HF. The suggested type strains, MK and HB, were typical members of the mesophilic and psychrophilic groups, respectively. It also showed that the species Chromobacterium viscosum Grimes (No. 25 in Appendix II), C. iodinum Davis (No. 61) and C. ianthinum Gilman (No. 59) were only distantly related to species of Chromobacterium as herein defined. However, the relations between the two species of Chromobacterium and Serratia and Pseudomonas were surprising. As shown in the diagram below, these four groups appeared to be roughly equally related to one another taxonomically. These points are also well shown in a model of the taxonomy (Lysenko and Sneath, 1959).



Strain TI is Chromobacterium ianthinum Gilman (No. 59)
Strain GR is Chromobacterium viscosum Grimes (No. 25)
Strain TE is Chromobacterium iodinum Davis (No. 61)

One might therefore consider whether the two species of Chromobacterium should be raised to the rank of genera, a point that Leifson (1956b) has also discussed. But one could with equal logic reduce the genera Serratia and Pseudomonas to species. There seems to be no answer to this problem at present, and I feel that we have scarcely begun to classify bacteria in a logical manner. It is likely, however, that the numerous families of the order Eubacteriales should be reduced to a few families, and a preliminary survey of the Eubacteriales (Sneath and Cowan, 1958) suggests that this may indeed be necessary. The survey confirms that the two groups of Chromobacterium should be classified together with the other Gram-negative rods (in what may be loosely called the Eubacteriales) but implies very extensive revision of the families, genera, and species within this order. The survey also showed a rather closer relation than is shown above of C. iodinum Davis to the mesophils, and the reason for this is obscure. I therefore feel that it is premature to attempt to classify the genus in any greater detail, and at the moment simply propose that it be included as a genus of the order Eubacteriales, with two species representing the mesophils and the psychrophils. Descriptions of these are given later in this chapter.

It is nevertheless of interest to note a few resemblances between Chromobacterium and some other genera which may suggest useful comparisons in later taxonomic studies.

Relation to pseudomonads. The chromobacteria resemble pseudomonads in possessing polar flagella and in the oxidative attack on glucose shown by psychrophils (see Entner and Stanier, 1951). However, the occurrence of bacteria like Aeromonas (Kluyver and van Niel, 1936) which are intermediate in many ways between the Pseudomonadaceae and the Enterobacteriaceae suggests that these families are not sharply distinct from one another (see Stanier and Adams, 1944; and Sneath, 1957b).

Relation to the Enterobacteriaceae. The psychrophils show less affinity to enterobacteria than mesophils, but the presence of lateral flagella and pigmentation suggest affinities with Serratia, which is now generally included in the Enterobacteriaceae, since it has O antigens in common with Escherichia (Kauffmann, 1949). The pattern of glucose fermentation in mesophilic chromobacteria seems very like that in Serratia (see Borman, Stuart, and Wheeler, 1944), where a "mixed acid" fermentation is found.

Relation to other genera. It is possible that some strains currently classified as Achromobacter or Flavobacterium are nonpigmented or yellow pigmented forms of Chromobacterium. Unfortunately these genera have taken on the function once fulfilled by Bacterium as convenient pigeon-holes for poorly-studied Gram-negative rods, and are in great confusion. Chromobacterium resembles Vibrio in its poor viability in culture and when freeze-dried; also the rods are often somewhat curved. The flagellation resembles that in some vibrios and nitrogen-fixing bacteria (Leifson and Hugh, 1953; Gray and Smith, 1950; Anderson, 1955). The mesophils resemble Rhizobium in their high lipoid and poly-β-hydroxybutyrate content (Burdon, 1946; Forsyth et al., 1958).

Description of the genus

Class SCHIZOMYCETES von Naegeli, 1857 Bericht über die Verhandlung der bot. Section d, 33 Versammlung deutscher Naturforscher u. Arzter. Botanischer Zeitung, p. 760.

Order EUBACTERIALES Buchanan, 1917 Journal of Bacteriology Vol. 2, p. 162.

Salient features of the genus Chromobacterium: Nonsporing Gram-negative rods, motile by both polar and peritrichous flagella. They produce the violet pigment violacein, grow on ordinary peptone media, are heterotrophic but with simple nutritional requirements, and inhabit soil and water.

Genus Chromobacterium Bergonzini 1880 Annuario della Società dei Naturalisti in Modena, ser. 2, Anno 14, parte Scientifica p. 153, parte Officiale p. 37, Emendavit Buchanan, 1918 Journal of Bacteriology, Vol. 3, p. 52, nomen generis conservandum Judicial Commission 1958a; 1958b; Opinion 16, International Bulletin of Bacteriological Nomenclature and Taxonomy, Vol. 8, pp. 137, 151.

Etymology. Greek noun chroma, colour and Greek noun bacterium, small rod; Modern Latin neuter noun, Chromobacterium, a coloured rod.

Synonymy. In the synonymy given below the page numbers in brackets refer to pages where bacteria recognizable as Chromobacterium are described among bacteria which are not of the genus Chromobacterium (in those instances where the generic name used by the author covers a large section of the work in question).

Chromobacterium Anonymous, 1881, p. 285; Buchanan, 1918, p. 52; 1925, p. 258; Winslow et al., 1920, p. 209; Holland, 1920, p. 222; Enlows, 1920, p. 28; Grimes, 1930, p. 381; Hauduroy et al., 1937, p. 88; Bergey et al., 1939, p. 92; Breed et al., 1948, p. 231; Magrou and Prévot, 1948, p. 105; Prévot, 1948, p. 16; Corpe, 1953, p. 470; 1954, p. 11; Leifson, 1956a, p. 60; 1956b, p. 339; Sneath, 1956a, p. 65; 1956b, p. 95; 1958, p. 131; Eltinge, 1956, p. 139; 1957, p. 37; Lysenko and Sneath, 1959, p. 290.

Chromobacterium in part Bergey et al., 1923, p. 117; 1926, p. 123; 1930, p. 157; 1934, p. 169; Ford, 1927, p. 468; Topley and Wilson, 1929, Vol. 1, p. 398; 1936, p. 493; Wilson and Miles, 1946, Vol. 1, p. 631; 1955, Vol. 1, p. 726; Krassilnikov, 1949, p. 480 (p. 581); Gilman, 1953, p. 48; Breed, Eltinge and Tobie in Breed et al., 1957, p. 292.

Not Chromobacterium Gieszczykiewicz, 1939, p. 21.

Cromobacterium [sic] Bergonzini, 1879, pp. 38, 39 in part; 1880, p. 153; Zimmermann, 1880, p. 1529.

Chromobacter Morris, 1954, p. 107.

Chromococcus Anonymous, 1881, pp. 285, 495; Buchanan, 1925, p. 258.

Cromococcus [sic] in part Bergonzini, 1879, p.32; 1880, p.150; Zimmermann, 1880, p.1529.

Bacterium in part Zopf, 1883 (p.68); 1885 (p.68); Schroeter, 1886, p.155 (p.157); de Lagerheim, 1891, p.77; Lehmann and Neumann, 1896, Vol.2 (p.264); 1899, Vol.2, p.185 (p.262); 1920, Vol.2 (p.403); 1927, Vol.2 (p.463); Chester, 1897, p.53 (p.117 Nos.293-295, 297, 298); 1801, p.117 (p.179); Migula, 1900 (pp.339, 491); Wolff, 1911, p.643; Cunningham and Raghavachari, 1924, p.1285; Enderlein, 1925, p.280; Cruess-Callaghan and Gorman, 1935, p.216.

Bacillus in part Flügge, 1886 (p.291); Eisenberg, 1888 (p.8); 1891 (pp.81, 91, 420, 421); Macé, 1889, p.528; 1897 (p.853); 1901 (pp.918, 920); 1913, Vol.2, (pp.412, 414); de Toni and Trevisan in Saccardo, 1889 (p.978); Trevisan, 1889, (p.18); Zimmermann, 1890 (p.84 (36); 1893 (p.94); Jordan, 1890 (p.838); Lustig, 1890 (p.88); 1893 (pp.75, 103); de Lagerheim, 1891, p.77; Roux, 1892 (pp.301, 307, 308); Sternberg, 1893 (pp.621, 631, 634, 641); Voges, 1893, p.302; Kruse in Flügge, 1896, Vol.2 (pp.311-313); Horrocks, 1901) (p.68); Chester, 1901, p.199 (pp.262, 305, 306); Matzuschita, 1902 (pp.50, 136, 138, 140, 168, 238); Miquel and Cambier, 1902 (pp.687, 690, 695); Minett, 1913, p.44; Bampton, 1913, p.129; Lehmann and Neumann, 1920, Vol.2, p.476; Calderini, 1925, p.765; Godfrin, 1934 passim; Waeldele, 1938, passim; Bergey et al., 1939 (p.703); Breed et al., 1948 (p.720, var. of B. polymyxa); Krassilnikov, 1949, p.560 (p.634).

Bacteridium in part Schroeter, 1872, p.126.

Bactrinium in part Fischer, 1903, p.60 (p.148).

Pseudomonas in part Migula, 1894, p.237; 1895, p.29; 1900, p.875 (pp.939, 941-944); 1901 (pp.378, 380, 382-385); Chester, 1901, p.306 (p.317); Pribram, 1933, p.4 (p.49); Krassilnikov, 1949, p.328 (pp.386, 387, 389).

Micrococcus in part Cohn, 1872, p.146 (p.157); Winter in Rabenhorst, 1881, p.42 (p.44); Flügge, 1883 (p.100); Grove, 1884 (p.9); Cornil and Babes, 1890, Vol.1, p.146; Lustig, 1890 (p.61); 1893 (p.36); Roux, 1892 (p.289); Bergonzini, 1892, p.173 (p.200); Sternberg, 1893 (p.601); Migula, 1900 (p.186); 1901 (p.128); Miquel and Cambier, 1902 (p.666); Calderini, 1925, p.773; Ford, 1927 (p.434).

Mikrococcus in part Adametz, 1888 (p.34); Matzuschita, 1902 (p.436).

Mikrokokkus in part Frosch and Kolle in Flügge, 1896, Vol.2 (p.181); Eisenberg, 1891 (p.42).

Streptococcus in part Trevisan, 1889 (p.31); de Toni and Trevisan in Saccardo, 1889 (p.1067); Chester, 1901 (p.70).

Aerobacillus in part Donker, 1926 (p.142).

Arthrobacterium in part de Lagerheim, 1891, p.77.

Mycobacterium in part Krassilnikov, 1941, p.89 (p.102); 1949, p.151 (p.171).

Description. When examined by the techniques described here and by Sneath (1956b), the members of the genus have the following characters, or the great majority of them.

Gram-negative or sometimes weakly Gram-positive bacteria, rod-shaped or coccobacillary, sometimes slightly curved, with little pleomorphism, measuring 0.5-1.1 x 1-6μ, not acid-fast, usually showing

barred or bipolar staining, nonsporing, though sometimes showing spore-like vacuoles, nonencapsulated (though intercellular slime may be present), usually containing visible fat droplets, sometimes with metachromatic or Gram-positive polar granules. They are usually motile by both polar and lateral (peritrichous) flagella which differ antigenically: the polar flagellum is single, up to 6μ long, with wavelength of c. 2.0μ and amplitude c. 0.35μ and is formed in all media; the lateral flagella are multiple, up to 10μ long, with wavelength c. 1.5μ and amplitude c. 0.4μ and are produced in solid media but seldom in liquid media; some strains lack lateral flagella.

The bacteria grow readily on the usual peptone media on which they may give a gelatinous rubbery growth, produce a violet ring of growth in broth and grow on potato. They produce in aerobic cultures a violet pigment, violacein, which is soluble in ethanol but not in water or chloroform and which in ethanol shows an absorption maximum at c. 580 mμ and a minimum at c. 430 mμ, with lesser maxima at c. 257 and c. 375 mμ and a minimum at c. 340 mμ. The pigment becomes green in 10% sulfuric acid in ethanol and then shows an absorption maximum at c. 700 mμ with lesser maxima at c. 660 and c. 413 mμ and with minima at c. 337 and c. 500 mμ. The pigment becomes green with caustic alkalis, rapidly becoming reddish-brown.

The bacteria show the usual resistance of vegetative bacteria to heat. and are not obligate halophils and are not highly salt-tolerant, are penicillin-resistant, die out readily in culture and when freeze-dried, and utilize citrate and ammonia as sole sources of carbon and nitrogen without requiring accessory growth-factors. They seldom utilize malonate. They are aerobic, sometimes facultatively anaerobic; they are psychrophilic or mesophilic but not thermophilic, and have optimum pH for growth between 7.0 and 8.0. They may produce hydrogen cyanide, and are highly sensitive to peroxides, thus showing the "catalase effect." They produce acidity but no gas from glucose and from some other carbohydrates but commonly acid is produced in small amount and commonly not anaerobically.

They are moderately or weakly proteolytic, may produce haemolysis, or turbidity from egg yolk, and are indole-negative, M.R.-variable, V.P.-negative, NH_3-positive, H_2S-negative, or doubtful, catalase-positive, phosphatase-positive, urease-negative, arylsulfatase-negative, and phenylpyruvate-negative. They may reduce methylene blue, usually reduce nitrates to nitrites and usually destroy nitrites (sometimes producing visible gas). The gluconate test is variable. They may be chitinolytic.

The bacteria are found commonly in soil and fresh water with a world-wide distribution: some strains are pathogenic and cause a septicaemic disease in man and animals. There are two distinct species. The type species is Chromobacterium violaceum Bergonzini, 1880 emend. Sneath, 1956a nomen typicum designatum Judicial Commission 1958a; 1958b.

Key to the species of Chromobacterium

I. Grow at 37° but not at 4°.
Produce hydrogen cyanide.
Give turbidity from egg-yolk.
Produce acid from trehalose.
Do not hydrolyze aesculin.
Produce no acid from arabinose, xylose or mannitol.
Moderately proteolytic, haemolytic and chitinolytic.

1. Chromobacterium violaceum
(Mesophils)

II. Grow at 4° but not at 37°.
Do not produce hydrogen cyanide.
Do not give turbidity from egg-yolk.
Do not produce acid from trehalose.
Hydrolyze aesculin.
Produce slight acid from arabinose, xylose and mannitol.
Poorly proteolytic, haemolytic and chitinolytic.

2. Chromobacterium lividum
(Psychrophils)

Species 1. Chromobacterium violaceum Bergonzini 1880 Annuario della Società dei Naturalisti in Modena, Ser. 2, Anno 14 parte Scientifica p. 153, parte Officiale, p. 37, emendavit Sneath, 1956a, International Bulletin of Bacteriological Nomenclature and taxonomy, Vol. 6, pp. 77, 79 nomen typicum designatum Judicial Commission, 1958a, 1958b, Opinion 16, ibidem vol. 8, pp. 137, 152.
(The mesophilic group of chromobacteria).

Etymology. Latin adjective violaceus, violet coloured.

Synonymy. (see Nos. 78-91 in Appendix II). Chromobacterium violaceum Martin, 1931, p. 68; Floch and de Lajudie, 1943, p. 2; Gilman, 1953, p. 48; Sneath et al., 1953, p. 276; Audebaud et al., 1954, p. 416; Sippel et al., 1954, p. 468; Darrasse et al., 1955, p. 704; Sneath, 1955, p. (i); Sippel, 1955, p. 1; Sneath, 1956b, p. 95; 1958, p. 137; Sneath and Cowan, 1958, p. 555; Lysenko and Sneath, 1959, p. 290; Joubert and Nguyen-Van-Liem, 1957, p. 341; Eltinge, 1957, p. 43 and vars. violaceum, anitritum, and purpureum. Chromobacterium violaceum var. manilae Schattenberg and Harris, 1942, p. 509. Chromobacterium manilae Leifson, 1956a, p. 60; 1956, p. 399. Chromobacterium laurentium Leifson, 1956a, p. 60; 1956b, p. 399. Chromobacterium ianthinum Bergey et al., 1939, p. 93; Breed et al., 1948, p. 233. Chromobacterium janthinum Breed, Eltinge and Tobie in Breed et al., 1957, p. 295; Eltinge, 1957, p. 42 and vars. janthinum, anitritum and purpureum. Chromobacterium amethystinum Gilman, 1953, p. 48. Chromobacterium violaceum Laurentium Ford, 1927, p. 470. Chromobacterium violaceum Manilae Ford,

1927, p.471. Chromobacter violaceum Morris, 1954, p.109. Bacterium violaceum Lehmann and Neumann, 1899, Vol.2, p.262; Cunningham and Raghavachari, 1924, p.1285. Bacterium Laurenti [sic] Enderlein, 1925, p.281. Bacterium janthinum Lehmann and Neumann, 1896, Vol.2, p.264; Cruess-Callaghan and Gorman, 1935, p.216. Bacterium violaceus [sic] Laurentius [sic] Chester, 1897, p.117. Bacillus violaceus Eisenberg, 1888, p.8; 1891, p.91; Chester, 1901, p.262; Ramchandani, 1930, p.957; Black and Shahan, 1938, p.1270; Soule, 1939, p.592; Dodd, 1941, p.19. Bacillus violaceous [sic] Lesslar, 1927, p.28; Minett, 1913, p.44. Bacillus violarius acetonicus Bréaudat, 1906, p.879. Bacillus violaceus acetonicus Lehmann and Neumann, 1920, Vol.2, p.476. Bacillus polymyxa variety Bergey et al., 1939, p.703; Breed et al., 1948, p.720. Bacillus violaceus Laurentius Jordan, 1890, p.838; Roux, 1892, p.308; Sternberg, 1893, p.631; Kruse in Flügge, 1896, Vol.2, p.312; Matzuschita, 1902, p.136; Calderini, 1925, p.767; Godfrin, 1934, pp.90, 231. Pseudomonas Laurentia Migula, 1900, p.944; 1901, p.384. Pseudomonas laurentia Krassilnikov, 1949, p.387. Pseudomonas janthina Chester, 1901, p.317 in part. Pseudomonas manilae Krassilnikov, 1949, p.389. Aerobacillus violarius Donker, 1926, p.142. Bacille bleu, van der Sleen, 1894, No.26. Violetter bacillus, Eisenberg, 1886, Tab.2, No.4. Bacille violet pathogène, Gauducheau, 1907, p.278.

Not Chromobacterium violaceum Ford, 1927, p.469; Leifson, 1956a, p.60; 1956b, p.399; Eltinge, 1957, p.41. Not Chromobacterium ianthinum Gilman, 1953, p.48. Not Chromobacterium amethystinum Holland, 1920, pp.217, 222; Bergey et al., 1926, p.126; 1930, p.161; 1934, p.173; 1939, p.94; Breed et al., 1948, p.232; Breed, Eltinge and Tobie in Breed et al. 1957, p.294; Krassilnikov, 1949, p.501. Not Bacillus violaceus Bampton, 1913, p.129. Not Pseudomonas ianthina Migula, 1900, p.941; Krassilnikov, 1949, p.387.

Neotype strain: Strain MK (Sneath, 1956b), (National Collection of Type Cultures 9757; American Type Culture Collection 12472) Sneath, 1956a, p.77; Judicial Commission, 1958a; 1958b, Opinion 16. This strain is a typical mesophilic strain, belonging to Leifson's subgroup 1a (see Leifson, 1956b and Chapter I).

Description. Bacteria having the features of the genus and usually also those listed below.

Young cells are small in size about 0.5-0.9 x 1.5-3.0μ, and contain abundant fat. Grow at 37° but not at 4°, are markedly proteolytic, give a positive egg yolk reaction, produce much hydrogen cyanide, and do not yield gelatinous colonies. They produce acid (usually both aerobically and anaerobically) but no gas from glucose. They produce moderate acid in peptone water from glucose, mannose, fructose, trehalose and sometimes from sucrose, starch, glycogen, and dextrin; not from salicin, dulcitol, mannitol or the other commonly-employed carbohydrates. They usually digest chitin. They do not hydrolyze aesculin. They grow moderately well anaerobically. Usually haemolytic on blood agar. Citrate is often utilized only slowly. Some strains are pathogenic for mammals.

Variation. Some strains do not produce acid from glucose anaerobically, and may then show less acid aerobically from the other carbohydrates which this species attacks, and may grow poorly anaerobically. Nonpigmented variants commonly arise in culture. Strains without lateral

flagella, and strains which do not attack nitrates or chitin also occur. The atypical mesophils which do not ferment glucose anaerobically are given as a distinct species by Leifson (1956a; 1956b; see No. 80) but it is possible that the other aberrant characters, e. g. poorer anaerobic growth, scanty fat droplets, inability to oxidize mannose, are caused by a single block in carbohydrate metabolism, such as loss of an enzyme which phosphorylates monosaccharides. At present I consider all the mesophils to belong to a single species, and find no sharp subgroups which require subspecific names.

Species 2. Chromobacterium lividum (Eisenberg, 1891 Bakteriologische Diagnostik 3e Aufl. p. 81) Holland, 1920 Journal of Bacteriology, Vol. 5, pp. 219, 222, syn. Bacillus janthinus Plagge and Proskauer, 1887, Zentralblatt für Hygiene und Infektions-Krankheit, Vol. 2, pp. 463, 464.
(The psychrophilic group of chromobacteria. For reasons for considering lividum to be the correct specific epithet see No. 94 in Appendix II).

Etymology. Latin adjective lividus, leaden-coloured, dark blue.

Synonymy (see Nos. 92-112 in Appendix II). Chromobacterium lividum Sneath 1956a, p. 78; 1956b, p. 95; 1958, p. 140; Sneath and Cowan, 1958, p. 555; Lysenko and Sneath, 1959, p. 290; Eltinge, 1957, p. 42 and vars. lividum and mesophilum. Chromobacterium amethystinum Holland, 1920, pp. 217, 222; Bergey et al. 1926, p. 126; 1930, p. 161; 1934, p. 173; 1939, p. 94; Breed et al., 1948, p. 232; Breed, Eltinge and Tobie in Breed et al. 1957, p. 294; Krassilnikov, 1949, p. 501. Chromobacterium amethystium [sic] Bergey et al., 1923, p. 121. Chromobacterium bamptonii Bergey et al., 1923, p. 119; 1926, p. 124; 1930, p. 159; 1934, p. 171. Chromobacterium cohaerens Grimes, 1930, p. 383. Chromobacterium membranaceum Bergey et al., 1923, p. 119; 1926, p. 125; 1930, p. 159; 1934, p. 171. Chromobacterium membranaceum amethystinum Ford, 1927, pp. 472, 473, 474. Chromobacterium violaceum Ford, 1927, p. 469; Leifson, 1956a, p. 60; 1956b, p. 399; Eltinge, 1957, p. 41 and vars. violaceum and mesophilum. Chromobacterium sp. Corpe, 1953, p. 470. Bacterium amethystinum Migula, 1900, p. 491; Chester, 1901, p. 179. Bacterium amethystinus [sic] Chester, 1897, p. 117. Bacterium jochromum de Lagerheim 1891, p. 77. Bacterium lividum Migula, 1900, p. 399. Bacterium lividus [sic] Chester, 1897, p. 117. Bacterium membranaceum amethystinum Cruess-Callaghan and Gorman, 1935, p. 216. Bacillus amethystinus Kruse in Flügge, 1896, Vol. 2, p. 312; Macé, 1897, p. 856; Matzuschita, 1902, p. 238; Calderini, 1925, p. 769; Chester, 1901, p. 262. Bacillus ianthinus Lustig, 1893, p. 76 in part. Bacillus janthinus Plagge and Proskauer, 1887, p. 464; Macé, 1889, p. 528; 1897, p. 855; 1901, p. 920; 1913, Vol. 2, p. 414; Zimmermann, 1890, pp. 84 (36), 140 (92); 1893, Taf. II. fig. 13; Eisenberg, 1891, p. 420 in part; Godfrin, 1934, pp. 95, 234 in part. Bacillus janthinus var. butyricus Deshusses and Novel, 1939, p. 7. Bacillus lividus Eisenberg, 1891, p. 81; Roux, 1892, p. 303; Sternberg, 1893, p. 621; Zimmermann, 1893, p. 94; Chester, 1901, p. 262; Matzuschita,

1902, p.140; Calderini, 1925, p.769; Godfrin, 1934, pp.97, 235; Krassilnikov, 1949, p. 634. Bacillus pseudolividus Matzuschita, 1902, p.168; Calderini, 1925, p.769. Bacillus membranaceus amethystinus Eisenberg, 1891, p.421; Sternberg, 1893, p.634; Miquel and Cambier, 1902, p.695; Bampton, 1913, p.129; Godfrin, 1934, pp.103, 237. Bacillus membranaceus amethystinus mobilis Germano, 1892, p.518. Bacillus violaceus Bampton, 1913, p.129. Bacillus violaceus diffusus Ajtai, 1897, p.666. Pseudomonas amethysina Migula, 1900, p.944; 1901, p.385; Krassilnikov, 1949, p.387. Pseudomonas ianthina Migula, 1900. p.941; Krassilnikov, 1949, p. 387 in part. Micrococcus violaceus Sternberg, 1893, p.601; Miquel and Cambier, 1902, p.666. Mikrococcus [sic] violaceus Adametz, 1888, p.34; Matzuschita, 1902, p.436. Mycobacterium ammethystinum [sic] Krassilnikov, 1941, p.102. Mycobacterium amethystinum Krassilnikov, 1949, p.171. Bacille violet, Macé, 1888c, p.526. Violet Bacillus, Ward, 1898, p.59.

Not Chromobacterium violaceum Bergonzini, 1880, p.153 emend. Sneath, 1956a, p.79; Martin, 1931, p.68; Floch and de Lajudie, 1943, p.2; Gilman, 1953, p.48; Sneath et al., 1953, p.276; Audebaud et al., 1954, p.416; Sippel et al., 1954, p.468; Darrasse et al., 1955, p.704; Sneath, 1955, p.(i); Sippel, 1955, p.1; Sneath, 1956a, pp.77, 79; 1956b, p.95; 1958, p.137; Sneath and Cowan, 1958, p.555; Lysenko and Sneath, 1959, p.290; Joubert and Nguyen-Van-Liem, 1957, p.341; Eltinge, 1957, p.43. Not Chromobacterium amethystinum Gilman, 1953, p.48. Not Bacillus violaceus Eisenberg, 1888, p.8; 1891, p.91; Chester, 1901, p.262; Ramchandani, 1930, p.957; Black and Shahan, 1938, p.1270; Soule, 1939, p.592; Dodd, 1941, p.19. Not Pseudomonas janthina Chester, 1901, p.317.

Proposed neotype strain. Strain HB (Sneath 1956b) (National Collection of Type Cultures 9796; American Type Culture Collection 12473) Sneath, 1956a, p.79. The Judicial Commission (1958a; 1958b, Opinion 16) did not act on the proposal to designate this as neotype strain, but this typical psychrophilic strain may profitably be considered as an unofficial neotype.

Description. Bacteria having the features of the genus and usually also those listed below.

Young cells are large, measuring about 1.0-1.2 x 2.5-6μ, and contain little fat. Grow at 4° but not at 37°, are poorly proteolytic, produce no egg-yolk reaction, produce no hydrogen cyanide, and commonly yield gelatinous rubbery colonies. They do not produce acid from carbohydrates anaerobically, but aerobically produce a small amount of acid from glucose, fructose, mannose, L(+) arabinose, D(+) xylose, mannitol, m-inositol, and often cellobiose, glycerol, galactose, sucrose, sorbitol, and maltose, not in salicin or trehalose; lactose is acidified late. Aesculin is hydrolyzed. Growth anaerobically is scanty or absent. Usually not haemolytic on blood agar. Citrate is rapidly utilized.

Variation. Nonpigmented variants occur, and variants which do not attack nitrate, which lack lateral flagella, which do not hydrolyze aesculin, and which give a gluconate reaction may be found. More rarely strains which do not acidify glucose or which are chitinolytic may occur.

CHAPTER XIX

CHROMOBACTERIOSIS : HISTORY AND CLINICAL FEATURES

Introduction

Although scarcely euphonious, the term chromobacteriosis (coined in its French form by Nguyen-Van-Liem, 1957, see Joubert and Nguyen-Van-Liem, 1957) is a convenient name for the disease caused by infection with strains of Chromobacterium, a disease which these authors have well reviewed. In most of the literature on infections by chromobacteria the red and yellow chromogens are also included, and these cases have been reviewed, though very incompletely, by Wheat, Zuckerman and Rautz (1951), Patterson, Banister and Knight (1952) and Vernon and Hepler (1954). This course has something to be said for it as a practical measure, and it is instructive to compare the infections by other chromogens with those by Chromobacterium. Such a comparison is briefly given below. Nevertheless, it does not seem to me to be wise to extend the term chromobacteriosis to include infections by these other bacteria and even infections by Whitmore's bacillus, as Brisou (1958) has suggested.

Infections by Serratia strains. These infections are not uncommon. Wheat et al., (1951) note that Serratia most commonly causes urinary infections, but occasionally bronchitis, pneumonia, septicaemia, endocarditis, and meningitis. Unlike Chromobacterium it shows no special tendency to cause lymphadenitis or liver abscesses. There is a belief that infections are often due to secondary invasion after overdosages with multiple antibiotics, to which Serratia is often very resistant (Anonymous, 1952a, 1952b). Red sweat is probably not usually caused by Serratia (Powell and Sharvill, 1954). The history of Serratia infections goes back to the last century, and some of the earlier records are those of Ferchmin (1892), du Bois Saint-Sévrin (1894, whitlows in fishermen), Bertarelli (1903a, 1903b), Fortineau (1904, p.103 who lists a number of early records), and Woodward and Clarke (1913). Some recent reports are those of Lane (1935, septicaemia complicating agranulocytosis), Paine (1946, aerosol bronchitis), Gurevitch and Weber (1950, urinary infection), Levy-Zaks (1954, meningitis), Hawe and Hughes (1954, endocarditis), Robinson and Woolley (1957, pseudohaemoptysis), Gale and Lord (1957, pseudohaemoptysis) and Papapanagiotou and Aligizakis (1959, empyaema). Strains may sometimes be pathogenic for insects (Steinhaus, 1941) and a similar organism which infects lizards has been reported under the name of Serratia anolium (Clausen and Duran-Reynals, 1937; Duran-Reynals and Clausen, 1937). A number of strains of Serratia have been found to be virulent for laboratory animals (e. g. Rosahn and Hu, 1933; Aitoff, Dion and Dobkevitch, 1936). Fulton, Forney and Leifson (1959), in an excellent study of the group, list a number of additional cases of infection.

Pseudomonas infections. Pseudomonas aeruginosa is well known to be occasionally pathogenic. The human cases have been excellently reviewed by Stanley (1947), who noted that while secondary infection of local lesions is very common, yet systemic infections can occur. The latter are usually in children, or in debilitated subjects, have a high

mortality and commonly show "ecthyma gangrenosa" and other gangrenous lesions. Eye infections, meningitis, and bronchopneumonia also occur. Strains of Pseudomonas may be pathogenic for small mammals and reptiles (Caldwell and Ryerson, 1940), and insects (Bucher and Stevens, 1957). Some strains virulent for animals can also be virulent for plants (Elrod and Bruan, 1942), a most unusual behaviour, though this also occurs with Sporotrichum (Benham and Kesten, 1932). It is possible that the causative organism of melioidosis, Whitmore's bacillus, should be regarded as a species of Pseudomonas. The case of violet pus described by Uyeno (1923) was caused by Pseudomonas aeruginosa.

Miscellaneous pathogenic chromogens. Besides chromogenic staphylococci and mycobacteria a few other chromogens may occasionally cause disease. Waisbren (1951) mentions a number, and others are those recorded by Pangalos (1929) and Castellani (1955) (axillary dematosis by Micrococcus violagabriellae, No.42 in Appendix II). It is doubtful if the violet chromogen isolated from the eye by McFarlane (1895, 1897, p.525) was a true pathogen (see No.68 in Appendix II).

Records of Chromobacterium infections

The first record appears to be that of Woolley (1904, 1905) of septicaemic infection in buffaloes. The first human case is probably that of Lesslar (1927). All the records of which I know are listed in Table 14. Stitt, Clough and Branham (1948, p.146) mention local abscesses caused by Chromobacterium but cite no reports.

Darrasse et al., (1955) draw attention to the close resemblance of chromobacteriosis to melioidosis. Both are uncommon septicaemic or pyaemic diseases caused by organisms commonly found as saprophytes in water (see Chambon, 1955) and evidently both organisms are of low infectivity in nature. Epizootics of melioidosis as well as of chromobacteriosis can occur in pigs (Nguyen-Ba-Lyong, 1956; Sippel et al., 1954). However, the two organisms are quite distinct both culturally and antigenically.

Epidemiology

These infections seem to be restricted to tropical or subtropical climates. This is probably because mesophilic strains are uncommon in temperate climates, and there is therefore less chance of infection.

Human cases. There do not appear to be any special features of the cases in humans with regard to age, race or occupation, though information is scanty. Males are affected more commonly than females. The avilable data are noted in Table 15.

Animal cases. There is very little information on these except for swine. However, cases in buffaloes have been recorded several times and these animals, like swine, may be particularly susceptible, perhaps on account of their love of wading in stagnant water. Woolley (1904, 1905) reported that his three cases were sporadic ones from widely separated herds, and not from an epizootic. Although no cases have been reported in birds, Sippel et al. (1954) found a high titre of agglutinins in the serum of a hen from a farm where an outbreak had occurred in swine. There

Table 14. Records of infection by Chromobacterium. Expanded from Sneath et al. (1953)

Record Number	Year	Author	Place and Country	Host
1	1904	Woolley (1904, 1905)	Manila, P.I.	Buffalo, 3 cases
2	1922	Broudin (1922)	Saigon, Indochina	Wild boar
3	1927	Lesslar (1927)	Kuala Lumpur, Malaya	Man
4	1931	Martin (1931)	Kuala Lumpur, Malaya	Man
5	1931	Martin (1931)	Kuala Lumpur, Malaya	Man
6	1933	L.S. da Silva *	Penang, Malaya	Man
7	1933	L.S. da Silva *	Penang, Malaya	Man
8	1937	Black and Shahan (1938)	Florida, USA	Man
9	1938	Sartory et al., (1938a, 1938b)	Strasbourg, France	Man
10	1939	Soule (1939)	Florida, USA	Man
11	1940	Schattenberg and Harris (1941)	New Orleans, USA	Man
12	1942	Floch & de Lajudie (1943)	French Guiana	Bull
13	1949	Sippel (1955, p.11, (case 3 of 1949)	Georgia, USA	Pig
14	1951	Sneath et al., (1953, No.11); Case 1 in this study.	Singapore, Malaya	Man
15	1952	Sneath et al., (1953, No.12); Case 2 in this study.	Taiping, Malaya	Man
16	1952	Sneath et al., (1953, No.13); Case 3 in this study.	Taiping, Malaya	Man
17	1952	H.D.G. Hetherington * (No.14 in Sneath et al., 1953) Case 4 in this study.	Taiping, Malaya	Man
18	1952	Sippel et al. (1954, Reeves case).	Georgia, USA	Cow
19	1953	Sippel et al. (1954, Sealy case, 1 in Sippel 1955, p.9)	Georgia, USA	Swine, many cases.
20	1954	Audebaud et al., (1954)	Brazzeville, French Equatorial Africa	Monkey
21	1954	Darrasse et al., 1955	Dakar, French West Africa	Man
22	1955	Sippel (1955, p.12, Case 4, Porter case)	Georgia, USA	Swine, possibly several cases.
23	1957	J.P.F. Whelan *	Singapore, Malaya	Monkey
24	Not stated	Joubert & Nguyen -Van-Liem (1957)	Saigon, Indochina	Buffalo, 2 cases.
25	1958	Ellis and Schroeder (1958)****	Georgia, USA	Swine, possibly several cases.
26	1559	C.S. Roberts*	Alabama, USA	Swine, several cases.

* Unpublished, personal communication.

** The case notes were lost during the Japanese occupation of Malaya.

*** These strains died out in subculture and were not deposited in any culture collection (personal communications from Floch, Hetherington, Sippel, and Audebaud.

(record no.)	Form of disease	Outcome	Strain letters
1	Regional adenitis and septicaemia	Death in all three	
2	Septicaemia	Death	
3	Pyaemia with liver abscesses	Death	
4	Pyaemia with liver abscesses	Death	
5	Urinary infection; no necropsy	Death	
6	Pyaemia with liver abscesses **	Death	
7	Local subcutaneous abscess **	Not recorded	
8	Skin lesions, later pyaemia	Death (Soule, 1939)	SH
9	Dental abscess, but possibly not authentic case (see Waeldele, 1938, p. 55)	Not recorded	
10	Regional adenitis and pyaemia	Death	
11	Local lesion leading to septicaemia	Death	
12	Bronchopneumonia	Death	Strain lost***
13	Pneumonia	Death	Strain lost***
14	Urinary infection; Case notes in Protocol 20.	Not recorded	BH
15	Regional adenitis later pyaemia with liver abscesses; Case notes in Protocols 21-25.	Death	BN
16	Mild diarrhoea (organism isolated from stools: case notes in Protocol 26).	Recovery	Strain lost***
17	Transient rectal bleeding and diarrhoea (organism isolated from stools; case notes in Protocol 27).	Recovery	Strain lost***
18	Pyaemia	Death	RV
19	Epidemic of about 60 cases, regional adenitis, septicaemia and pneumonia.	Death in most	SL
20	Hepatic abscesses	Death	Strain lost***
21	Hepatic abscesses	Death	DK
22	Cervical adenitis and lung abscesses.	Death	PT
23	Pet monkey thought to have died of rabies, pure culture of Chromobacterium from brain but no rabies virus.	Death	BM
24	Not recorded	Death	
25	Cervical adenitis and lung abscesses	Death	
26	Cervical adenitis and lung abscesses	Death in some	

**** Ellis, E.M. and W.F. Schroeter. 1958. Chromobacterium violaceum infection in swine. Vet. Med., 53, 578-579.

Table 15. Age, sex, race, and occupation in human cases of chromobacteriosis.

Number in Table 14	Author	Age in years	Sex	Race	Occupation
3	Lesslar	Elderly	M	Chinese	-
4	Martin	-	M	European	-
5	Martin	-	M	-	-
8	Black and Shahan	6	M	European	-
10	Soule	15	F	-	-
11	Schattenberg and Harris	21	M	Negro	-
14	Sneath et al.	20	M	European	Soldier
15	Sneath et al.	25	M	European	Soldier
16	Sneath et al.	20	M	European	Soldier
17	Hetherington	35	M	European	Soldier
21	Darrasse et al.	44	M	European	Timberyard worker: a diabetic

- = not recorded.

are no records of infection in insects (Steinhaus, 1946) and the organism is harmless if fed to cockroaches (Longfellow, 1913).

Swine appear to be particularly susceptible, and most of our knowledge is due to the excellent work by Sippel. He observed an epidemic in pigs in which over half the herd died within a few weeks (the Sealy outbreak, No.19 in Table 14 described by Sippel et al., 1954 and Sippel, 1955). He also found that cross-infection did not occur between infected and healthy pigs kept in the same pen, so it is not highly contagious.

Portal of entry of the organism. In most cases this is unknown. There was good evidence in the case of Schattenberg and Harris (1941) and in Case 2 (No.12 in Sneath et al., 1953) that entry was through a skin lesion. In the former it was apparently a thorn wound in the foot, in the latter an abrasion on the thigh. Darrasse et al. (1955) found no wound in their patient, and thought that a bowel infection was more likely, since Sneath et al. (1953) had reported the organism in faeces of patients (Cases 3 and 4; Nos.16 and 17 in Table 14). The case of Soule (1939) started as a cervical abscess nine months before the onset of septicaemia. Cervical adenitis has been noted in swine, which can be infected by mouth (Sippel et al., 1954), so it is possible that there are other routes of infection besides wounds. Indeed the evidence of Sippel and his colleagues is greatly in favour of entry through the pharyngeal tissues in swine. Similar behaviour is found with anthrax in swine, and also melioidosis (Nguyen-Ba-Luong, 1956), suggesting that swine may be rather generally susceptible to transpharyngeal invasion. Sippel (1955, pp.9-15) was able

occasionally to infect swine with infected drinking water, and the water supplying the swine affected by the epidemic came from ponds, swamps, and wells; from one pond he isolated a strain very similar to that from the infected pigs.

Clinical features

Signs and symptoms. Both in man and animals the presenting features of the cases have generally been those of a septicaemia. However, it is likely that mild infections without septicaemia can occur. Sneath et al. (1953) reported two cases of intestinal infection in which the only features were mild diarrhoea with or without transient rectal bleeding: these are Cases 3 and 4 in this study (Nos. 13 and 14 in Sneath et al., 1953 and Nos. 16 and 17 in Table 14). The stools gave heavy growths of Chromobacterium, but the patients soon recovered. Short clinical notes are given in Protocols 26 and 27 in Appendix I. Martin (1931) observed one case of urinary infection, and my colleagues and I reported another (Case 1 in this study, No. 11 in Sneath et al., 1953; No. 14 in Table 14). In the former, death was probably due to heart failure unconnected with the infection. The records of the latter could not be traced and the few details known are given in Protocol 20. The case No. 9 in Table 14 may not have been genuine, since Waeldele (1938, p. 55) was uncertain whether the organism came from the dental abscess, and no clinical details were reported. One of da Silva's cases (No. 7 in Table 14) was a localized abscess, but the outcome is not known. Sippel et al. (1954) mention that some pigs evidently had mild infections consisting of only cervical adenitis, and soon recovered.

In the more severe infections the disease has sometimes started as a lymphadenitis. In the case of Soule (1939) it was a cervical abscess, clinically tuberculous, but yielding Chromobacterium on culture: this abscess was incised, but did not heal, and nine months later the patient died of septicaemia. In the case of Black and Shahan (1938) the primary lesion may have been the inguinal adenitis which occurred some months before the patient was first seen. Probably Case 2 (No. 12 in Sneath et al., 1953, No. 15 in Table 14) also commenced as an inguinal adenitis arising from an infected sore, with septicaemia subsequently. Presumably the course of events in the case of Schattenberg and Harris (1941) was similar, starting in a thorn wound of the foot.

In the outbreak in pigs reported by Sippel et al. (1954) acute cervical lymphadenitis was usually present early in the disease, while in the monkey described by Audebaud et al. (1954) the animal had had some sores several days before the onset. Except in the case of Soule, these local lesions were acute inflammatory ones, often containing pus.

Other cases have been presented as septicaemia without evidence of prior lesions (Lesslar, 1937; Martin, 1931; Darrasse et al., 1955) or, in animals, as pneumonia (Floch and de Lajudie, 1943; Sippel's case 3 in Sippel, 1955, p. 11, No. 13 in Table 14). The buffaloes reported by Woolley died suddenly without obvious signs of previous illness.

If the local lesions do not subside, the disease continues as a septicaemia with pyogenic manifestations. Most authors have reported high fever, prostration, anorexia, rigors, delirium and some have noted an

enlarged or tender liver with mild icterus and pulmonary congestion or diffuse pulmonary consolidation due respectively to liver abscesses and lung abscesses or bronchopneumonia. There is a moderate neutrophilic leucocytosis. The fever is variously described as high, hectic or swinging, and the upper level has been usually 104° to 105°F with a pulse-rate of 120 to 140 per minute. The respiration rate is also raised moderately, with dyspnoea in cases showing pulmonary involvement. The urine is generally normal or shows only a trace of albumin. Schattenberg and Harris and Darrasse et al. obtained positive blood cultures in their patients. Diarrhoea, usually not bloodstained, and rapid loss of weight have also been reported. In pigs the chief clinical signs were cervical adenitis, loss of weight, anorexia, coughing and dyspnoea with a "double expiratory lift" (Sippel et al., 1954). There would seem to be no special clinical features to differentiate the disease from other septicaemic conditions, and the hepatic and pulmonary signs and symptoms seem to be due simply to the occurrence of multiple abscesses, consolidation or pyogenic effusions. However, it is noteworthy that cerebral or renal abscesses, and clinical signs of these, have not been reported. In one case pyaemic skin lesions have been found: Black and Shahan reported widely scattered anthrax-like lesions, consisting of a dark brown eschar from 1.5-5 cm in diameter surrounded by pustules with a distinct violet tinge. These lesions healed very slowly. Soule (1939), in a further report on Black and Shahan's case, mentions that when the case relapsed similar lesions again appeared. In one case of Martin (No.5 in Table 14), which was a urinary infection, there were ascites, hepatomegaly, cardiac failure and joint pains, but no autopsy was made and these were probably unconnected with the infection.

Course of the disease. The septicaemic cases have had a high mortality. Only one case, that of Black and Shahan, recovered from the acute phase, after being critically ill for several weeks, but 13 months after this a relapse occurred with cervical adenitis, skin lesions and high fever, and death occurred soon after (reported by Soule, 1939). No autopsy was made but there seems little doubt that this was the same infection as the previous illness. In this case the skin lesions on healing showed a violet pigmentation of the scars. This was also noted in one of my cases (No.15 in Table 14, see Protocol 22) but whether this is significant is uncertain.

In the other septicaemic cases death has occurred within a week or ten days (except Case 2, No.15 in Table 14, in which it is believed that the infection was temporarily checked by antibiotics). The cases of Schattenberg and Harris (1941) and Darrasse et al. (1955) are recorded as dying in circulatory collapse. Most cases were before the advent of the tetracycline antibiotics, and the treatment then available, chiefly sulfonamides and blood transfusion, have been quite ineffective, though Black and Shahan thought that the latter may have given some benefit. As mentioned in the next chapter, the tetracyclines would seem the best drugs to use, as penicillin is ineffective.

Personal observations

The case notes, post-mortem reports and bacteriological investigations on the four cases which I have studied are given in detail in Appendix I in Protocols 20 to 27. These were also reported briefly by Sneath et al. (1953). They are summarized below.

Case 1 in this study (No.11 in Sneath et al., 1953, No.14 in Table 14 and see Protocol 20 in Appendix I). This was a case of urinary infection, which is thought to have cleared up rapidly: unfortunately the case notes could not be traced. Chromobacterium strain BH was isolated in pure culture from the urine.

Case 2 in this study (No.12 in Sneath et al., 1953, No.15 in Table 14, the source of the strain BN). A young man developed a sore on the thigh and subsequent inguinal adenitis: He was admitted with fever, neutrophil leucocytosis, and a slightly enlarged and tender liver and diagnosed as amoebic hepatitis (although no amoebae were ever found) and treated with emetine, chloroquine and aureomycin (chlortetracyclin). He improved, but when these drugs were stopped he relapsed and did not respond to emetine and chloroquine. His liver became very large and pus was aspirated from it: this gave a pure growth of Chromobacterium. There was a high fever and he died soon after. Necropsy (see Protocol 22) showed multiple abscesses in the liver, and subpleural abscesses of the lung, which yielded pure cultures of the same organism. Evidently the aureomycin had suppressed the infection (the strain was sensitive to this antibiotic), and the failure to respond to treatment during the relapse was due to the fact that treatment with this drug was not recommenced (see case notes in Protocol 21). The strain which was isolated, strain BN, was highly pathogenic for laboratory animals (see Protocols 24 and 25). The abscesses were acute pyogenic lesions consisting of necrosis with many Gram-negative bacilli and a cellular reaction in which polymorphonuclear leucocytes predominated (see Protocol 23 and Figure 12). At post-mortem it was thought at first to be melioidosis, but no organisms other than Chromobacterium were found in a very thorough investigation, and it seems quite clear that this was the causative organism.

Case 3 in this study (No.13 in Sneath et al., 1953, No.16 in Table 14). This was a young man who had mild diarrhoea, malaise and slight leucocytosis and slight fever with some liver tenderness. No amoebae were found but he was treated as a case of amoebic hepatitis and soon recovered. His stools yielded a heavy and almost pure growth of a mesophilic Chromobacterium, but this strain was later lost. A few weeks later it was not found in the stools (see Protocol 26).

Case 4 in this study (No.14 in Sneath et al., 1953, No.17 in Table 14, clinical summary provided by Dr. H.D.G. Hetherington, see Protocol 27). This was a man who had diarrhoea with a little blood in the stools, and the case was suggestive of amoebiasis. He was treated for this but some months later was admitted with acute diarrhoea, of which he had several bouts, with visible blood in the stools, which gave a heavy growth of a mesophilic Chromobacterium. He responded well to aureomycin (chlortetracyclin) and the organism disappeared from the stools. Later he developed frank amoebiasis which was also successfully treated. This strain of Chromobacterium was also lost.

CHAPTER XX

CHROMOBACTERIOSIS : PATHOLOGY, DIAGNOSIS AND TREATMENT

Morbid anatomy and histology

Early lesions. The only case where early lesions were examined was that of Soule (1939, No.10 in Table 14). The initial cervical adenitis showed on biopsy a gross and microscopic appearance of mixed tuberculous and pyogenic infection. At necropsy a similar picture was found in the lung lesions (Dr. C.V. Weller, in Soule, 1939). Tubercle bacilli were never found, and despite the marked difference from the acute pyogenic inflammation reported in the other cases, it is likely that Soule's case was not a mixed infection with tuberculosis, since Sippel (1955, p.35) has reported that chronic lesions in swine are grossly and histologically similar to tuberculosis. Sippel observed caseation, epithelioid cells and sometimes giant cells.

Appearance at autopsy. The chief lesions are seen in the liver and lungs. In some (e.g. the cases of Lesslar, 1927 and Darrasse et al., 1955) the liver has been mainly involved with little change in the lungs. In others (e.g. the pigs studied by Sippel et al., 1954, the buffaloes of Woolley, 1904, 1905, the bull of Floch and de Lajudie, 1943) the lungs and cervical lymphnodes have been the sites of abscesses and the liver showed little change. In others (e.g. Case 2 in this study, No. 12 in Sneath et al., 1953 and the case of Schattenberg and Harris, 1941) both liver and lungs showed multiple abscesses. Abscesses have also been reported in the spleen (Soule, 1939; Sippel et al., 1954) and in the skin (Black and Shahan's case reported by Soule, 1939). A pleural exudate has been present in the cases with lung lesions, and this has varied from sero-sanguinous fluid to thick pus and fibrin.

The liver abscesses vary in size, evidently depending upon their age. Usually they have been firm, irregularly rounded, slightly raised, and yellowish, from 2 to 15 mm in diameter, the larger ones containing thick yellowish-white pus. In case 2 (No.12 of Sneath et al., 1953) there were also some older abscesses, several centimetres in diameter, lined by a rough fibrinous pseudomembrane. The hepatic abscesses, if present, are usually very numerous and distributed throughout the organ: they show the distribution of haematogenous abscesses.

The lung lesions again vary in size from 2 to 20 mm, and are generally discrete yellowish abscesses or areas of advanced consolidation, patchily scattered through the lungs (but more frequent at the bases in humans) and usually of an irregularly rounded shape, slightly raised and firm.

In those cases which commenced with a regional lymphadenitis, the lymph nodes have generally shown small necrotic areas containing thick yellowish pus, but the actual site of entry where known has usually shown little abnormality. The spleen has seldom shown frank abscesses, but is usually large, soft and friable. A small amount of clear pericardial effusion is often reported.

The other organs have seldom shown any gross changes other than mild congestion or cloudy swelling such as may be attributed to septicaemia. In particular there are no abscesses in brain or kidneys. The adrenals may contain haemorrhagic areas (Darrasse et al., 1955). An abscess in the wall of the stomach was found in Case 2 (No. 12 in Sneath et al., 1953, see Protocol 22).

Healed lesions. Sippel (1955, p. 10) has observed healed lesions in swine which recover from the infection. These are fibrotic nodules in the lungs, which resemble the scars of healed tubercles both macroscopically and microscopically.

Histology. There is general agreement that the predominating histological lesion is necrosis, which seems always to be marked in the abscesses (Fig. 12, p. 394). Surrounding the necrosis, which is often of a caseous type, is an area of congestion and oedema with a mixed neutrophilic, lymphocytic, plasma cell and macrocytic cellular response, which in the older lesions may be replaced by a fibrinous area containing many epithelioid cells. In pigs the lesions have shown fewer neutrophils than the human cases (Sippel, 1955, p. 38). This basic histological picture is seen in lesions in all organs and is well illustrated by the figures of Schattenberg and Harris (1942) and Darrasse et al. (1955). In the case of Darrasse et al. (1955) there was marked fatty change in the liver, but this was attributed to diabetes mellitus, from which the patient was known to have suffered. The more chronic lesions may show a histological appearance similar to tuberculosis (Sippel et al., 1954; Sippel, 1955, p. 35).

Bacteriology of the lesions. Mesophilic strains of Chromobacterium are readily isolated from the abscesses in the liver, lungs, and other organs. Authors have usually reported pure cultures and when tubercle bacilli, yeasts, fungi, and anaerobes were looked for they have never been found. In a few cases the organism has been isolated before death from pus of the abscesses (Black and Shahan, 1938; Soule, 1939; Case 2 (No. 12 of Sneath et al., 1953) and from blood cultures (Schattenberg and Harris, 1941; Darrasse et al., 1955). Where there has been pleural effusion, pneumonia or acute adenitis the organism has also been generally isolated from these lesions, and usually also from the spleen even though no abscesses were present in this organ. Shahan (1943) noted that no contaminants were present in cultures from skin lesions, and attributed this to a bacteriostatic effect of the organism, but Woolley (1904, 1905) noted some contaminants (Bacillus subtilis and staphylococci) in one of his cases. Microscopically, bacteria have generally been readily seen in the pus as Gram-negative rods, occurring singly, sometimes slightly curved and sometimes showing bipolar staining.

Pathogenesis. The multiple abscesses in lungs and liver are quite sufficient to explain the serious course of most of these cases. It seemed possible that the production of hydrogen cyanide by the bacterium (see Chapter XIII) might be a factor in causing death, but an examination of the blood of a rabbit immediately after death from septicaemia showed no increase over the normal level in blood cyanide plus thiocyanate by methods of Aldridge (1944) and Epstein (1947) (F. E. Buckland, personal communication). The natural infection is presumably caused by the invasion by an unusually virulent strain in an unusually susceptible individual

(except perhaps in swine) but the cases are not usually in debilitated individuals as is implied by Joubert and Nguyen-Van-Liem (1957).

Summing up, one may briefly describe the morbid anatomy as showing multiple abscesses in the liver and lungs, predominantly in the former organ in man and in the latter organ in pigs, which consist of necrotic areas surrounded by a mixed cellular response, and which contain the bacteria in large numbers. The primary lesion may sometimes be in a wound, or perhaps in the pharynx or bowel, and regional acute pyogenic lymphadenitis may then be also found. This picture is very like melioidosis, tularemia, Pasteurella pseudotuberculosis infections, and bovine necrobacillosis (see Darrasse et al., 1955; Schattenberg and Harris, 1941; Jensen, Flint and Griner, 1954; Wilson and Miles, 1948, Vol. 2, pp. 1415, 1649, 1720).

Personal observations. The necropsy findings in Case 2 (No. 12 in Sneath et al., 1953, no. 15 in Table 14) are given in detail in Protocol 22 in Appendix I. They may be summarized as follows. The liver contained about a dozen large abscesses 2.5-5 cm in diameter, containing greenish pus and lines by a rough fibrinous pseudomembrane, and also many smaller abscesses scattered throughout the organ. The gall bladder was normal. There were many subpleural abscesses at the bases of the lungs, with patches of overlying fibrinous exudate. The spleen and para-aortic lymph nodes were enlarged and soft. There was an intramural abscess of the stomach. The other organs showed no important changes: the ulcer on the thigh was almost healed and the regional inguinal lymph nodes were normal. The typical histological picture was found in the affected organs (see Fig. 12 and Protocol 23).

A mesophilic strain of Chromobacterium, strain BN, was readily isolated from the pus of the abscesses in the liver and lungs and from the spleen pulp by plating onto blood agar. No other organisms were found (see Protocol 24).

Reproduction of the disease in animals

The fact that many mesophilic strains of Chromobacterium are virulent for laboratory animals has been discussed earlier. Most workers have succeeded in reproducing features of the natural disease in animals, though with some difficulty. Woolley (1904, 1905) found that large doses of culture killed guinea pigs and rabbits by septicaemia within a day or two. Smaller doses sometimes produced regional lymphadenitis, a congested, necrotic and "gelatinous" local lesion, with occasional abscesses in liver, lungs, and spleen, and sometimes miliary abscesses also. This is not unlike the natural disease. However, Minett (1913), Black and Shahan (1938), Floch and de Lajudie (1943) and Darrasse et al. (1955) were unable to reproduce the liver abscesses, as their animals always died of acute septicaemia. Schattenberg and Brown (1941) found that their strain would invade through wounds in the feet of mice, and cause regional adenitis, and usually septicaemia. Both in mice and rabbits they sometimes found abscesses in the liver and lungs after infection through a wound. Audebaud et al. (1954) reproduced the disease in a monkey, Cercopithecus cephus, which died with liver and lung abscesses. Joubert and Nguyen-Van-Liem (1957) had similar findings on infecting a

monkey (Macacus cynomolgus). Sippel et al. (1954), Sneath et al. (1953) and Joubert and Nguyen-Van-Liem (1957) also could reproduce the abscesses in rabbits and guinea pigs by injection of small doses, while large doses caused death from septicaemia within 24 hours. One of Sippel's animals (a pig) showed the unusual features of abscesses in the heart, tongue, skin and kidney (Sippel, 1955, p. 25).

Most workers have been unable to infect rats, mice, rabbits or guinea pigs by mouth (Woolley, 1904, 1905; Sneath et al., 1953; Darrasse et al., 1955) but this seems to be due to the animals which were used, for Sippel and his colleagues were able to infect swine by mouth, and to reproduce the cervical adenitis and pneumonia seen in the natural infections. It was also possible (Sippel, 1955, pp. 15-19) to infect pigs by the intranasal, intraperitoneal, intravenous and intratracheal routes, causing pneumonia and septicaemia.

In the animals which die of septicaemia within 24 hours there are no obvious changes at autopsy, other than a diffuse congestion of the skin and abdominal organs and some oedema and necrosis at the site of inoculation. Woolley found more oedema in rabbits and more necrosis in cats than in the other animals he used. The usual laboratory animals are susceptible to infection and probably mice are the most sensitive (see p. 325) though they usually die of septicaemia. The animal in which it is easiest to reproduce the pyaemic disease seems to be the rabbit.

The experience of my colleagues and myself has been that it is easy to reproduce septicaemia in guinea pigs and rabbits with large inocula and pyaemia with small inocula of virulent strains (see p. 326). In Protocols 24 and 25 some such experiments are described in detail. After intraperitoneal or subcutaneous inoculation in the flank there are commonly multiple abscesses in the liver, and occasionally peritonitis or a localized abscess at the site of inoculation with enlarged regional lymph nodes. After intravenous inoculation both liver and lung abscesses are commonly found. Mr. G. J. Harper in 1952 inoculated the strain BH into two rabbits intravenously, using 1 ml of a broth culture. One rabbit died on the 6th day, and at post-mortem there were a few abscesses in the liver and many in the lungs. The second rabbit died on the 7th day; on the 6th day a blood culture yielded the organism. Post-mortem examination also showed abscesses in liver and lungs. Ellis and Schroeder (1958) noted caseous pancreatic lesions in mice.

Diagnosis

Chromobacteriosis is a pyogenic infection, producing lymphadenitis, septicaemia, pyaemia, or less often urinary and bowel infections, and it shows no characteristic clinical or pathological features. The diagnosis can therefore only be made by cultivation of the responsible organism. This has always been a mesophilic strain of Chromobacterium which grows well at 37° (except for the doubtful case No. 9 in Table 14). The culture of aspirated pus is the best means of diagnosis, and the organism is readily isolated by plating onto aerobic blood agar plates and incubating at 37°. It is unsafe to use plain nutrient agar plates since small inocula may not grow (see p. 282). Blood cultures and exudates should be similarly examined. The pale violet shiny convex colonies seen after 18 hr at 37° (darker after 2 days) are very characteristic. Urine or faeces

may be plated on MacConkey agar or desoxycholate citrate agar plates as well as on blood agar, since the organism grows well on these selective media, giving colourless colonies which soon produce the characteristic violet pigment. This pigment enables immediate identification of the bacterium. On anaerobic plates growth occurs but no pigment is formed. The diagnosis has rarely been made before death and almost every author has commented upon the likelihood of the organism being dismissed as a contaminant. It is probable that these infections are more common than they appear. If an infection by a nonpigmented strain should ever occur, it will be difficult to identify, though the production of hydrogen cyanide may be a clue.

Serology. Animals which recover from experimental infection show a raised titre of antibodies to the infecting strain (Woolley, 1904, 1905; Sippel et al., 1954). The titres recorded have ranged up to 2000. A titre of 1280 was found in the case of Black and Shahan (1938) on the 10th day of the illness. Martin (1931) found the titre with 'O' suspensions to be 280 and with 'H' suspensions 680. Sippel (1955, p. 63) observed that many sera from normal swine in Georgia, U.S.A. would agglutinate one of his strains, suggesting that mild infections may be common. In 519 pigs examined, titres of 80 or over were found in 11.6%. In three animals it was 320. However, it is likely that the antibodies are mainly strain specific (see p. 315) and many antigenic types may cause infections, so the main use of serology in diagnosis is confirmatory. Agglutination titres for other bacteria (such as Brucella and enteric organisms) have not been found to be raised.

Differential diagnosis. Chromobacteriosis is unlikely to be confused with any other disease if the organism is recovered by culture and recognized as a pathogen. Otherwise it may be confused with plague, tularemia, melioidosis, acute glanders, acute bacterial endocarditis, miliary tuberculosis, acute biliary sepsis and particularly acute amoebic hepatitis. In swine it may be confused with various forms of pneumonia, but in man it is hepatic lesions which predominate. A history of a recent wound with regional adenitis may be given. From endocarditis it should be distinguishable by the absence of cardiac signs, and from miliary tubercle by the character of the luecocytosis and the absence of miliary "mottling" in chest X-rays. Rarely the disease may be confused with pasteurellosis, pseudotuberculosis or necrobacillosis in animals, or with anthrax.

Prognosis and treatment

Prognosis. There is insufficient knowledge of the urinary and intestinal forms of the infection to say anything about prognosis, except that even if untreated it is probably good. The few cases of these forms appear generally to have recovered completely. In those cases which present as a localized lymphadenitis there is again little information, but the risk of septicaemia appears to be high. In septicaemic cases the prognosis is very grave. All the recorded human cases have died, either in a week or two, or after a relapse some months later, and without energetic antibiotic treatment this is much the most probable outcome. Sippel and his colleagues (1954) have observed what they believe to be

occasional cases of recovery in swine. With antibiotic treatment early cases will probably recover (see below).

Prophylaxis. The outbreaks in swine which are probably due to infected drinking water should be preventable by disinfection of water and other hygienic measures. It is inadvisable for people with open wounds to bathe in stagnant water in the tropics, since wounds may be a portal of infection. Sippel (1955, p.68) considers that there may be some small risk of human infection from infected pig carcasses, and that suspected carcasses should be destroyed. Such carcasses may only show healed lesions similar to healed tubercular lesions. Joubert and Nguyen-Van-Liem (1957) state that chromobacteriosis is not a scheduled disease in tropical countries, and there seems to be no need for quarantine or similar measures.

Immunity. Very little is known about this. Sippel (1955, p.53) attempted to immunize guinea pigs and swine with formolized cultures and culture filtrates. He thought that they afforded a little protection to challenge with living cultures. Dodd (1941, p.75) also found that culture filtrates afforded partial protection. Passive immunization of mice was not protective. Schattenberg (1940) and Schattenberg and Harris (1942) claimed that repeated injections of culture filtrates gave a high degree of protection of mice. Nothing is known about the degree of cross-protection between different strains.

Treatment. Before the advent of the tetracyclin antibiotics, no treatment was found to be of any great use. Sulfonamides are ineffective. Sippel attempted to treat the disease in swine (Sippel, 1955, pp.65-67) with aureomycin (chlortetracyclin) in doses of 12 mg/kilo intravenously every 24 hours. He found that this cut short the infection, but had to be continued for several days to prevent a relapse. The drug was also effective in guinea pigs. In Case 2 (No.12 in Sneath et al., 1953) there was evidence that the infection responded well to aureomycin, and relapsed when it was discontinued.

It has been shown earlier (Chapter VII) that strains of Chromobacterium are sensitive (to levels which are readily attained in the body) of streptomycin, aureomycin, terramycin and chloramphenicol. However, I have observed that streptomycin-resistant variants readily occur in vitro, so this drug is best avoided. The strains are always resistant to penicillin. It is clear that heavy and prolonged therapy with one of the other antibiotics is needed, and terramycin or aureomycin is probably the best. A careful watch for relapses should be made for several months at least. It may be advisable to use chloramphenicol together with a tetracyclin.

SUMMARY

1. The genus Chromobacterium consists of aerobic nonsporing Gram-negative rod-shaped bacteria which grow on ordinary peptone media and produce a violet pigment (violacein). This genus is described, together with the history of the genus, the nomenclature and the disease—chromobacteriosis—which is produced by infection with these organisms. A thorough review of the literature upon the blue and violet chromogenic bacteria is given in an Appendix.

2. The genus is sharply divisible into two groups, the mesophils and the psychrophils, which may conveniently be regarded as two species. The name Chromobacterium violaceum is the correct name f r the mesophilic group and Chromobacterium lividum appears to be the correct name for the psychrophilic group.

3. Forty-two strains of Chromobacterium have been studied (nineteen of them mesophils and twenty-one of them psychrophils) isolated from soil, water, and cases of chromobacteriosis. A number of other chromogenic bacteria were also studied and found not to belong tc the genus. Chromobacteria are inhabitants of soil and water and may be isolated by special techniques.

4. The bacteria are Gram-negative or Gram-variable rods, nonsporing, nonencapsulated and showing barred or bipolar staining. They are motile by both a single polar flagellum and multiple peritrichous flagella, which differ antigenically and in morphology. The latter type are only formed abundantly in cultures on solid media.

5. The bacteria grow on the usual media, giving round, convex violet colonies, sometimes of a gelatinous or rubbery consistency. In broth they give a violet ring of growth at the surface and they grow on potato but frequently form little pigment on this medium. Mesophilic strains liquefy gelatin stab cultures and coagulated serum.

6. They show the usual resistance to heat of vegetative bacteria. They are resistant to penicillin and to sulfonamides and are sensitive to streptomycin, chloramphenicol and the tetracyclins. They are very sensitive to traces of peroxides in culture media and therefore show the "catalase effect" i.e. small inocula are inhibited on such media and the inhibition is overcome by minute quantities of catalase. They rapidly die out in cultures. They are aerobic and sometimes facultatively anaerobic, and are not highly halo-tolerant. The optimum pH for growth is between pH 7 and 8. They have simple nutritional requirements.

7. The violet pigment violacein is described and compared with some other bacterial pigments. Violacein is formed only in aerobic cultures and nonpigmented variants are commonly seen in cultures. Violacein is soluble in ethyl alcohol but not in water or chloroform; in ethanolic solution the absorption spectrum shows maxima at about 375 and 580 mμ and a minimum at about 430 mμ. It has some antibiotic activity and is a complex indole derivative.

8. Carbohydrate fermentation reactions vary considerably. No gas is formed, but mesophils attack a few carbohydrates with the production of moderate amounts of acid and generally do so anaerobically, while psychrophils attack many carbohydrates but only aerobically and with the production of only a small amount of acid. The bacteria are indole negative, M-R variable, V-P negative, catalase-positive, H_2S-negative (or weak), NH_3-positive and urease-negative. They generally reduce

nitrate and destroy nitrite. Some other biochemical reactions are also described.

9. The antigenic structure has been investigated. Besides the two forms of flagellar antigens, they have somatic antigens of the O and R type. The O antigens tend to be strain specific. The mesophils and psychrophils form two distinct but heterogeneous serological groups.

10. Some mesophilic strains, whether from cases of chromobacteriosis or from water, are virulent for laboratory animals. They do not produce a potent exotoxin. The evidence for an endotoxin is conflicting. The pathogenic strains do not form a distinct subgroup, culturally or serologically.

11. The genus Chromobacterium Bergonzini emend. Buchanan is included in the Order Eubacteriales Buchanan, with two species, Chromobacterium violaceum Bergonzini emend. Sneath (the mesophilic group) and Chromobacterium lividum (Eisenberg) Holland (the psychrophilic group). Type strains of the two species are described.

12. Mesophils (Chromobacterium violaceum) show a higher optimum temperature than psychrophils, and grow at 37° but not at 4°. Mesophils produce abundant hydrogen cyanide, which can readily be smelled in cultures, give a positive egg-yolk reaction, and are more strongly proteolytic, haemolytic, and chitinolytic than psychrophils. Mesophils produce acid from trehalose but not from arabinose, xylose, or mannitol, and they do not hydrolyze aesculin.

13. Psychrophils (Chromobacterium lividum) grow at 4° but not at 37°. They do not produce hydrogen cyanide, do not give a positive egg-yolk reaction, but they hydrolyze aesculin and produce a small amount of acid from arabinose, xylose, and mannitol and also from a wide range of other carbohydrates. They are feebly proteolytic, haemolytic, and chitinolytic.

14. Chromobacteriosis is a rare infection of man and animals in tropical climates caused by mesophilic strains of Chromobacterium. It is generally a septicaemic or pyaemic disease with a high mortality, probably contracted from contaminated water either through wounds or by mouth. It sometimes commences as a regional adenitis, and the main lesions are pyogenic abscesses in the liver and lungs, predominantly the liver in man and the lungs in swine. Among swine definite epidemics may occur, probably caused by contaminated drinking water.

15. The abscesses show much necrosis with a mainly neutrophilic cellular response, and contain large numbers of the bacteria which can be readily cultivated from them. Urinary or bowel infections or localized abscesses also occur. The disease can be reproduced in experimental animals by injecting pure cultures of Chromobacterium.

16. Four cases in man are described, one of urinary infection, two of transient bowel infection and one of regional adenitis developing into pyaemia with liver and lung abscesses leading to death. There was evidence that this case initially responded to aureomycin (chlortetracyclin) and relapsed when this drug was discontinued.

17. The diagnosis must be made by culture of the organism since there are no special features to distinguish chromobacteriosis from several other pyaemic diseases such as melioidosis which it closely resembles. The prognosis in systemic infections is grave, but in early cases heavy and prolonged administration of antibiotics, preferably one of the tetracyclins, offers a prospect of successful treatment.

APPENDIX I

TECHNICAL METHODS AND EXPERIMENTAL AND CLINICAL DATA

Protocol 1. Chromobacteria: size, shape and staining reactions.

The data are from films of cultures grown on nutrient agar for 18 hr. at 25°. The size and shape, and barred or bipolar staining are from films stained with Löffler's methylene blue. Fat was stained by the method of Burdon (1946) using Sudan Black B. Metachromatic granules were stained by the modification of Neisser's stain descrined by Sneath (1956b). Capsules were stained by Hiss' method and other films were stained by the methods of Gram, Ziehl-Neelsen, and Albert, as given by Mackie and McCartney (1948, pp. 82, 86, 88, 90, 94, 96). For Gram-staining, both the ethanol method of decolorizing followed by neutral red as counterstain, and the acetone method followed by safranine were used.

MESOPHILS (C. violaceum)

Strain	Size (μ)	Fat	Barring	Notes
FH	0.8 x 2	+++	+	g
MK	0.6 x 1.5	+++	+	g
BH	0.8 x 2	+++	+	G
BN	0.8 x 1.5	++	+	g
AM	1.0 x 5	+	++	G, ch
MW	0.8 x 3	++	+	g, V
TV	0.7 x 1.5	+++	+	
LG	0.6 x 1.5	+++	+	g
SL	0.7 x 2	++	+	G
RV	0.8 x 1.5	+++	+	
DK	0.6 x 1.5	+++	+	g, m
TA	0.7 x 1.5	+++	+	g
TB	0.6 x 1.5	+++	+	g
PT	0.8 x 1.5	++	+	g
LW	0.9 x 1.5	+	+	g
SH	0.9 1.5	+	+	ch
RT	0.9 x 2	+	+	ch
MH	0.8 x 2	+++	+	g
BM	0.7 x 2	+++	+	

PSYCHROPHILS (C. lividum)

Strain	Size (μ)	Fat	Barring	Notes
NT	0.9 x 4	+	+	g, ch
EA	1.1 x 4	+	+	
EB	1.0 x 4	+	++	G
EC	1.0 x 5	+	++	g, M, ch, V
GA	1.0 x 5	-	+	G, ch
DA	1.0 x 5	+	+	g, ch
NC	1.0 x 4	+	++	g, ch
MA	1.0 x 5	+	+	ch, V
MB	0.8 x 2	+	+	
MC	1.0 x 4	+	++	g, ch
HA	0.9 x 4	+	++	G, V
HB	1.0 x 3	+	++	g, m
HC	0.9 x 3	+	+	G
HD	1.0 x 4	+	+	g, ch
HE	1.0 x 3	+++	++	G
HF	0.9 x 3	+	++	G
IN	1.0 x 3	+	+	G, m, ch
CA	1.0 x 4	+	+	g, m
PB	1.0 x 4	-	++	g
RU	1.0 x 4	+	++	g
ED	1.0 x 4	+	+	
EF	1.0 x 3	+	+	g
BC	1.0 x 4	+	+	

OTHER BACTERIA

	Strain	Size (μ)	Fat	Barring	Notes
Chromobacterium viscosum	GR	0.8 x 1.5	+++	+	M, C, Gram-positive
Chromobacterium iodinum	TE	0.8 x 2	-	+	Gram-positive
Chromobacterium ianthinum	TI	0.9 x 2.5	++	+	
Serratia marcescens	NCTC 1377	0.6 x 1.0	-	-	
Serratia marcescens	NCTC 9493	0.7 x 1.0	-	-	
Pseudomonas aeruginosa	NCTC 2000	0.8 x 1.5	-	+	g
Pseudomonas aeruginosa	NCTC 6749	0.8 x 2	+	+	

Symbols: Fat: +++ = heavy fat staining, ++ = moderate, + = slight, with a few fat globules in the majority of organisms.
Barring: ++ = marked, + = slight, - = no visible barring. With very short organisms barring gives the appearance of bipolar staining.
Notes: G = Gram-positive polar granules seen.
M = metachromatic polar granules with Neisser's stain.
Ch = chains of 5 to 10 members frequently seen.
C = a capsule visible with Hiss' stain.
V = young cultures may be Gram-variable.
Lower case letters indicate that the feature is slight, or seen in few of the organisms. All strains were Gram-negative (or variable in young cultures) except GR and TE.

The staining methods are standard ones with the exception of Burdon's method. This method is as follows: air dried smears are lightly fixed by heat, and flooded with a 0.3% solution of Sudan Black B (G.T. Gurr Ltd., London) in 70% ethanol. They are left for 5 to 10 minutes and then blotted dry and the precipitated dye is removed by brief treatment with xylene. They are blotted dry and counterstained in 0.5% aqueous safranine for a few seconds, rinsed and blotted dry.

Protocol 2. Motility and flagellation.

The motility was examined in hanging drop preparations of nutrient broth cultures grown at 25° for 18 hours. The flagellation was examined in stained films of 18 hour growth at 25° on nutrient agar plates. Subpolar flagella have been scored as lateral if they had the other features of lateral flagella. The stain used was that of Leifson (1951).

MESOPHILS (C. violaceum)			
Strain	Motility	Polar flagella	Lateral flagella
FH	+	1, short, lw	none
MK	+	1, short, lw	3-5, long, sw
BH	+	1, short, lw	scanty, long, sw
BN	+	1, short, lw	3-8, long, sw
AM	+	1, long, lw	none
MW	+	1, short, lw	none
TV	+	1, long, lw	1-3, short, sw
LG	+	1, short, lw	3-6, long, sw
SL	+	1, short, lw	1-4, long, sw
RV	+	1, short, lw	1-3, long, sw
DK	+	1, short, lw	3-8, long, sw
TA	+	1, short, lw	scanty, long, sw
TB	+	1, short, lw	3-5, long, sw
PT	+	? none	1-3, long, sw
LW	+	1, short, lw	1-5, long, sw
SH	+	1, short, lw	2-5, long, sw
RT	+	1, short, lw	2-5, long, sw
MH	+	1, long, lw	none
BM		not examined	

PSYCHROPHILS (C. lividum)			
Strain	Motility	Polar flagella	Lateral flagella
NT	+	? none	4-8, long, lw
EA	+	1, short, lw	1-4, short, sw
EB	+	1, short, lw	scanty, short, sw
EC	+	1, long, lw	1-3, short, sw
GA	+	1, long, lw	scanty, short, sw
DA	+	1, short, lw	1-3, long, sw
NC	+	1, short, lw	1-4, long, sw
MA	+	1, short, lw	1-2, long, sw
MB	+	1, short, lw	1-2, long, sw
MC	+	1, long, lw	none
HA	+	1, short, lw	1-2, short, sw
HB	+	1, short, lw	1-3, short, sw
HC	+	1, short, lw	1-3, short, sw
HD	+	1, short, lw	1-5, short, sw
HE	+	1, short, lw	scanty, short, sw
HF	+	1, short, lw	none
IN	+	1, short, lw	scanty, short, sw
CA	+	1, short, lw	scanty, short, sw
PB	+	1, long, lw	1-3, long, sw
RU	+	1, long, lw	1-2, long, lw
ED	+	not examined	
EF	+	not examined	
BC	+	not examined	

OTHER BACTERIA

	Strain	Motility	Polar flagella	Lateral flagella
Chromobacterium viscosum	GR	-	none	none
Chromobacterium iodinum	TR	-	none	none
Chromobacterium ianthinum	TI	+	1-4, long, lw	none
Serratia marcescens	NCTC 1377	+	none	1-4, long, lw
Serratia marcescens	NCTC 9493	+	none	1-3, long, lw
Pseudomonas aeruginosa	NCTC 2000	+	1, long, lw	none
Pseudomonas aeruginosa	NCTC 6749	+	1, long, lw	none

Symbols. Under each type of flagella is given, first the number of flagella, then their approximate length (short is under about 8μ) and then the approximate wavelength (sw = a wavelength of 1 - 1.5μ; lw = a wavelength of 1.5-2.5μ). A number of slides were photographed and these parameters measured directly, but this was not done for all strains.

Protocol 3. Cultural characteristics: media used and appearance in gelatin stab cultures.

The media used routinely for growth varied with the laboratory in which the work was performed, but all strains were re-examined together upon the same media toward the end of the investigation. For these, Bacteriological Peptone from Evans Medical Supplies Ltd., Liverpool, was used as a base. The more important routine media and those used for studying the cultural appearances are given below. Other media (e.g. for biochemical tests) are given in the appropriate protocols; the same peptone was used also for these.

Nutrient broth. This was prepared according to the method of Hartley (1922).

Nutrient agar. This was the nutrient broth solidified by addition of 1.5% of Japanese agar.

Nutrient gelatin. 15 g of Bacto-Gelatin (Difco, Ltd.) was dissolved in 100 ml of the nutrient broth at 80°, and cleared by adding 5 ml of egg, keeping the temperature at 80° for about half an hour, and filtering off the coagulum. The pH was then adjusted to 7.2, and the medium sterilized by heating to 100° for 30 minutes on three successive days. This was used both for gelatin stabs and gelatin plates.

Potato slopes. Plugs of peeled fresh potato were placed in tubes resting on moist cotton-wool, and sterilized by steaming on three successive days.

Blood agar. This consisted of nutrient agar with addition of 5% of sterile defibrinated horse blood.

Löffler's inspissated serum slopes. These were prepared with sterile horse serum according to the method given in Mackie and McCartney (1948, p.163) and were inspissated at 85° for 2 hours.

Main features of growth in gelatin stab cultures incubated 7 days at 20°.

Strain	Degree of surface growth	Growth in stab: Degree	Growth in stab: Type	Liquefaction: Degree	Liquefaction: Type
		MESOPHILS (C. violaceum)			
FH	++	+	F	++	I
MK	+++	+	F	+++	I
BH	+++	++	F	+++	I
BN	+++	++	F	+++	I
AM	+++	+	F	++	I
MW	++	+	F	++	N
TV	+++	+	F	++	I
LG	+++	++	F	+++	I
SL	++	+	F	++	I
RV	+++	+	F	+++	I
DK	+++	++	F	+++	I
TA	++	++	F	+++	I
TB	+++	++	F	+++	I
PT	+++	++	F	++	I
LW	++	±	F	+	N
SH	++	+	F	+	I
RT	++	+	F	++	N
MH	++	±	F	++	N
BM	++	+	F	++	I
		PSYCHROPHILS (C. lividum)			
NT	+	-		-	
EA	++	-		±	C
EB	++	±	F	-	
EC	++	-		-	
GA	++	-		-	
DA	+++	-		-	
NC	++	±	F	-	
MA	++	-		-	
MB	++	-		-	
MC	++	±	F	-	
HA	++	-		-	
HB	+	-		-	
HC	+	-		-	
HD	+	-		-	
HE	++	±	F	-	
HF	++	-		-	
IN	++	-		-	
CA	+	-		-	
PB	+	±	F	-	
RU	+	±	F	-	
ED	+	-		-	
EF	-	-		-	
BC	++	-		±	C

		OTHER BACTERIA				
		Degree of surface growth	Growth in stab: Degree	Growth in stab: Type	Liquefaction: Degree	Liquefaction: Type
Chromobacterium viscosum	GR	+++	±	F	++	S
Chromobacterium iodinum	TE	+++	±	F	++	S
Chromobacterium ianthinum	TI	+++	+	F	++	N
Serratia marcescens	NCTC 1377	+++	+++	F	+++	N
Serratia marcescens	NCTC 9493	+++	+++	F	+++	I
Pseudomonas aeruginosa	NCTC 2000	+++	-		+++	S
Pseudomonas aeruginosa	NCTC 6749	+++	-		+++	S

Symbols. F = filiform, I = infunduliform, N = napiform, C = crateriform, S = stratiform. ± to +++ = increasing degrees from barely visible to well-marked.

Protocol 4. Heat resistance tests

I. Heat resistance at 56°.

5 ml nutrient broth cultures grown for 2 days at 25° were well shaken, and heated in a water bath at 56° ± 0.2°. A loopful was removed to blood agar plates after 5, 10, 15, and 30 minutes. The broth heated for 30 minutes was removed, and added to 10 ml of sterile broth and, after incubation for 4 days at 25°, was plated out on blood agar. All plates were incubated for 4 days at 25°. The results were very uniform. Strains MK, BH, BN, LG, and PB showed a few survivors in a loopful after 5 minutes, but after 10 minutes there were no survivors of any strain. The broths heated for 30 minutes were all sterile. Strains BM, ED, EF, and BC were not tested.

II. Method of Bampton (1913).

The details of Bampton's method were followed as closely as possible. Five loopfuls of growth scraped off a 3-day culture (on nutrient agar at 20°) were emulsified in 50 ml of 0.85% sodium chloride, and 5 ml of this suspension were heated in tubes in a water bath for 30 minutes, one set of tubes at 42.5° ± 0.2° and another set at 45° ± 0.2°. 1 ml samples were then removed to pour plates of nutrient gelatin, which were incubated at 20° for 4 days and the colonies counted. When the plates showed many colonies, the approximate number was roughly estimated. The results are shown below.

Colonies in 1 ml of heated suspension plated in gelatin plates, after 4 days at 20°.

Strain	Suspension 42.5°	Heated at: 45°	Strain	Suspension 42.5°	Heated at: 45°
	MESOPHILS (C. violaceum)			PSYCHROPHILS (C. lividum)	
FH	Over 10^4	Over 10^4	NT	About 10^3	27
MK	Over 10^4	Over 10^4	EA	271	19
BH	Over 10^4	Over 10^4	EB	Over 10^4	Over 10^4
BN	Over 10^4	Over 10^4	EC	Over 10^4	Over 10^4
AM	Over 10^4	About 10^3	GA	Over 10^4	About 10^3
MW	Over 10^4	27	DA	Over 10^4	About 500
TV	Over 10^4	33	NC	Over 10^4	About 10^3
LG	Over 10^4	About 10^3	MA	About 10^3	41
SL	Over 10^4	About 10^3	MB	Over 10^4	Over 10^4
RV	Over 10^4	Over 10^4	MC	Over 10^4	Over 10^4
DK	Over 10^4	Over 10^4	HA	Over 10^4	Over 10^4
TA	About 10^3	20	HB	Over 10^4	Over 10^4
TB	Over 10^4	45	HC	Over 10^4	Over 10^4
PT	Over 10^4	Over 10^4	HD	Over 10^4	Over 10^4
LW	Over 10^4	23	HE	Over 10^4	Over 10^4
SH	Over 10^4	230	HF	Over 10^4	Over 10^4
RT	Over 10^4	21	IN	Over 10^4	Over 10^4
MH	Over 10^4	360	CA	Over 10^4	26
			PB	Over 10^4	About 10^3
			RU	Over 10^4	21

Protocol 5. Resistance to antibiotics.

Penicillin. Nutrient agar plates containing 100 international units/ml of sodium benzyl penicillin were streaked with a loopful of 48-hour nutrient broth cultures grown at 25°. The plates were incubated at 25° for 24 hours. All of the 38 mesophilic and psychrophilic strains of Chromobacterium listed in Table 1 grew well, as did strain TI. The Gram-positive organisms (GR and TE) did not grow.

Other antibiotics. Nutrient agar plates containing known concentrations in doubling dilutions were streaked with a loopful of broth cultures as described above and incubated for 24 hours at 25°. Nine mesophils (FH, MK, BH, BN, MW, TV, LW, SH, and LG) and one psychrophil (NT) were tested. The minimum concentration which completely inhibited growth is given below. Sulfadiazine: all grew on 100 μg/ml. Streptomycin: all were inhibited by 3 μg/ml. Chloroamphenicol: all were inhibited by 12 μg/ml, and all except FH, BH and NT by 6 μg/ml. Chlortetracyclin: all except LG and NT were inhibited by 0.25 μg/ml, and all grew at 0.125 μg/ml. LG and NT were inhibited by 0.5 μg/ml. Oxytetracyclin: All except NT were inhibited by 1.5 μg/ml. NT was inhibited by 6 μg/ml. Polymyxin E: LW and NT were inhibited by 25 μg/ml. The rest grew at 50 μg/ml.

Streptomycin resistant variants of strains MK, BN, and LG were readily obtained by plating about 10^9 organisms on agar containing 100 μg/ml of streptomycin: usually several colonies appeared after 2 days at 37°, which on subculture grew well in media containing this concentration of the drug.

Protocol 6. Temperature for growth.

A drop of a nutrient broth culture (grown for 2 days at 25°) was run down a nutrient agar slope which contained abundant water of condensation. The slopes were incubated for 7 days (for 4 days at 44°). The optimum temperature was estimated visually after 1 or 2 days of growth.

	Growth in 7 days (4 days at 44°)									Optimum
Strain	2°	10°	16°	20°	25°	30°	35°	37°	44°	(at 24 hrs.)
MESOPHILS (C. violaceum)										
FH	-	++	+++	+++	+++	+++	+++	+++	+	37°
MK	-	++	+++	+++	+++	+++	+++	+++	-	35
BH	-	-	++	+++	+++	+++	+++	+++	+	37
BN	-	-	++	+++	+++	+++	+++	+++	+	37
AM	-	++	+++	+++	+++	+++	+++	+++	-	30
MW	-	+	+++	+++	+++	+++	+++	+++	-	30
TV	-	++	+++	+++	+++	+++	+++	+++	-	30
LG	-	+	+++	+++	+++	+++	+++	+++	+	37
SL	-	±	+++	+++	+++	+++	+++	+++	-	37
RV	-	+	+++	+++	+++	+++	+++	+++	-	35
DK	-	-	++	+++	+++	+++	+++	+++	-	37
TA	-	++	+++	+++	+++	+++	+++	++	-	30
TB	-	++	+++	+++	+++	+++	+++	+++	-	35
PT	-	++	+++	+++	+++	+++	+++	+++	-	37
LW	-	+	+++	+++	+++	+++	+++	+++	-	30
SH	-	-	++	+++	+++	+++	+++	+++	-	35
RT	-	-	+++	+++	+++	+++	+++	+++	-	37
MH	-	+	+++	+++	+++	+++	+++	+++	-	30
BM	-	-	+++	+++	+++	+++	+++	+++	-	35
PSYCHROPHILS (C. lividum)										
NT	++	+++	+++	+++	+++	-	-	-		25
EA	±*	+++	+++	+++	+++	+	-	-		25
EB	+	+++	+++	+++	+++	++	-	-		25
EC	++	+++	+++	+++	+++	++	-	-		25
GA	+++	+++	+++	+++	+++	+++	+	-		30
DA	++	+++	+++	+++	+++	+++	-	-		30
NC	+	+++	+++	+++	+++	+++	-	-		30
MA	+	+++	+++	+++	+++	+++	-	-		30
MB	+	+++	+++	+++	+++	+++	-	-		30
MC	+	+++	+++	+++	+++	+++	±	-		30

Strain	2°	10°	16°	20°	25°	30°	35°	37°	44°	Optimum (at 24 hrs.)
HA	+	+++	+++	+++	+++	+++	-	-		30
HB	++	+++	+++	+++	+++	++	-	-		25
HC	++	+++	+++	+++	+++	-	-	-		25
HD	++	+++	+++	+++	+++	+++	-	-		30
HE	+++	+++	+++	+++	+++	+++	-	-		30
HF	++	+++	+++	+++	+++	+++	-	-		30
IN	+	+++	+++	+++	+++	++	±	-		30
CA	++	+++	+++	+++	+++	+	-	-		25
PB	++	+++	+++	+++	+++	+	-	-		25
RU	++	+++	+++	+++	+++	+++	-	-		30
ED	-	+++	+++	+++	+++	+++	++	-		30
EF	-	+++	+++	+++	+++	++	+	-		30
BC	++	+++	+++	+++	+++	+	-	-		25
OTHER BACTERIA										
GR	-	++	+++	+++	+++	+++	+++	+++	-	35
TE	-	++	+++	+++	+++	+++	+++	+++	-	35
TI	-	±	+++	+++	+++	+++	+++	+++	-	30

* Strain EA grew at 4° in 7 days.

Symbols. - = no visible growth; ± = growth visible with a hand lens; + to +++ increasing degrees of growth visible to the naked eye. Strains ED and EF gave erratic results on nutrient agar and the results shown are from tests on blood agar slopes, which gave reproducible results.

Protocol 7. X-ray crystallography of bacterial pigments.

These investigations were kindly performed by Professor J. A. C. Wilson of University College, Cardiff, and by Mrs. Olga Kennard, who made powder spectrographs of many bacterial pigments.

Violacein. The crystalline violacein from strains RV and NT appeared to be identical, and a sample of violacein kindly provided by Dr. J. S. Beer (which was the pigment studied chemically by him and his colleagues, see Chapter X) is probably identical with the other two. The powder patterns (Cu K α radiation and nickel filter) are shown below, giving the spacing in angstroms and the approximate intensity of the lines (s = strong, m = medium, w = weak, v = very).

Violacein samples: d(Å) and intensity

RV	NT	From Dr. Beer
12.58 w	12.98 w	
8.86 vw		
6.80 m (2)	6.82 w (2)	7.17 mw
6.03 m (3)	6.07 w (3)	6.10 mw
5.08 w	5.07 vvw	5.18 mw
4.75 w		4.76 mw
4.47 w	4.42 vvw	4.52 mw
3.95 m	3.95	4.10 m (2)
3.78 m	3.79 vw	
3.73 vw		3.57 mw
3.40 w		
3.16 s (1)	3.19 m (1)	3.18 vs (1)
		3.01 w
		2.61 w
		2.42 w
		2.16 vw

In addition some other pigments were examined and the main powder spectrum lines are given below (Cu K α radiation with nickel filter and the conventions used above).

Prodigiosin. A sample from Dr. Tanner, the material reported on by Morgan and Tanner (1955).

Pyocyanin dihydrochloride from Roche, Ltd., Welwyn Garden City.

Iodinin from "Chromobacterium iodinum" strain RE, recrystallized three times from chloroform.

Phenazine α carboxylic acid from Pseudomonas aureofaciens, kindly provided by Dr. W.C. Haynes.

d (Å) and intensity

Prodigiosin	Pyocyanin dihydrochloride	Iodinin	Phenazine α carboxylic acid
11.18 m (2)	9.34 s (1)	8.02 vs (2)	11.08 w
7.84 w	8.04 w	4.77 s	7.69 vs (1)
5.72 w	6.85 vvw	4.30 m	6.66 vs (2)
5.31 w	6.00 w	3.75 w	4.62 w
4.86 w	4.74 vw	3.46 vvs (1)	4.34 w
4.47 s (1)	4.45 vw	3.32 m	3.75 m
3.84 m (3)	3.53 vw	3.11 vs (3)	3.52 w
3.65 w	3.38 m (2)	2.77 m	3.34 s
3.32 w	3.24 w (3)	2.70 w	3.24 vs (3)
3.04 w	3.01 vw	2.54 w	2.95 w
	2.88 vvw	2.38 m	2.55 w
	2.72 vvw	1.994 m	2.10 w
		1.992 w	
		1.780 w	
		1.651 w	

Protocol 8. Carbohydrate fermentation reactions in peptone water.

The medium consisted of 1% peptone water containing 0.5% of sodium chloride, 1% of carbohydrate and 1% of Andrade's indicator (quoted in Mackie and McCartney, 1948, p.159 - a 1% solution of basic fuchsin in water just decolorized with N-NaOH). They were sterilized by steaming on three successive days. After inoculation they were incubated at 25° for 14 days, and read every few days.

Strain	Glucose	Fructose	Mannose	Maltose	Trehalose	Sucrose	Glycogen	Dextrin	Starch	Glycerol	Sorbitol	Sorbose
MESOPHILS (C. violaceum)												
FH	A	A	A	a	A	-	--	-	-	?	?	-
MK	A	A	A	a	A	-	-	-	-	?	?	?
BH	A	A	A	a	A	AL	AL	AL	al	?	?	?
BN	A	A	A	a	A	-	AL	AL	AL	?	?	?
AM	A	A	A	a	A	-	-	-	-	?	-	-
MW	A	A	A	a	A	-	-	-	-	?	?	?
TV	A	A	A	a	A	-	al	AL	al	?	?	-
LG	A	A	A	a	A	A	AL	AL	AL	?	?	?
SL	A	A	A	a	A	A	-	-	-	?	-	?
RV	A	A	A	?	A	A	-	-	-	?	?	-
DK	A	A	A	?	A	-	-	-	-	?	?	-
TA	A	A	A	al	A	-	-	-	-	?	?	-
TB	A	A	A	?	A	-	-	-	-	?	?	?
PT	A	A	A	?	A	-	AL	AL	AL	?	?	?
LW	A	A	a	al	A	-	-	-	-	?	?	-
SH	A	A	a	?	A	-	-	-	-	-	?	?
RT	A	A	a	a	A	-	-	-	-	?	?	-
MH	a	a	a	-	a	a	-	-	-	?	?	-
PSYCHROPHILS (C. lividum)												
NT	a	-	-	-	-	-	-	-	-	-	-	-
EA	A	A	AL	a	?	-	-	-	-	-	-	-
EB	a	a	a	-	-	-	-	-	-	-	-	-
EC	?	?	?	?	-	-	-	-	-	-	-	-
GA	?	?	?	?	-	?	-	-	-	-	-	-
DA	A	?	-	?	-	-	-	-	-	-	-	-
NC	?	?	?	?	-	?	-	-	-	-	-	-
MA	a	al	?	al	-	?	-	-	-	-	-	-
MB	?	al	?	-	-	?	-	-	-	?	-	?
MC	?	?	?	-	-	?	-	-	-	?	-	-
HA	-	?	-	?	-	-	-	-	-	-	-	-
HB	?	?	-	?	-	?	-	-	-	-	-	-
HC	?	a	-	?	-	-	-	-	-	-	-	-

Psychrophils (cont.)

Strain	Glucose	Fructose	Mannose	Maltose	Trehalose	Sucrose	Glycogen	Dextrin	Starch	Glycerol	Sorbitol	Sorbose
HD	-	?	-	?	-	?	-	-	-	-	-	-
HE	?	a	-	?	-	-	-	-	-	-	-	-
HF	-	?	-	-	-	?	-	-	-	-	-	-
IN	?	?	-	-	-	-	-	-	-	-	-	-
CA	?	?	-	-	-	?	-	-	-	?	-	-
PB	?	-	-	?	-	?	-	-	-	-	-	-
RU	-	-	-	-	-	?	-	-	-	-	-	-
OTHER BACTERIA												
GR	?	-	-	-	-	-	-	-	-	?	-	-
TE	-	-	-	-	-	-	-	-	-	-	-	-
TI	a	-	-	-	-	-	-	-	-	-	-	-
1377	A	A	A	A	A	A	A	AL	A	A	A	AL
9493	AG	AG	AG	AG	AG	AG	AL	AL	AL	AG	AL	a
2000	?	?	-	-	-	-	-	-	-	-	-	-
6749	-	-	-	-	-	-	-	-	-	-	-	-

Symbols. A = marked acidity, a = slight acidity, ? = doubtful acidity, - = no acidity. L or l = late acidity, appearing after 7 days or more.

Protocol 9. Carbohydrate fermentation reactions in Hugh and Leifson's medium.

The medium of Hugh and Leifson (1953) consists of 0.2% peptone, 0.5% NaCl, 0.3% K_2HPO_4, 0.3% agar, and 0.003% bromthymol blue, with 1% of carbohydrate added. The base was made up (using solid bromthymol blue, not an alcoholic solution) adjusted to pH 7.1, autoclaved and mixed with the requisite volume of a 10% Seitz-filtered solution of carbohydrate, and dispensed in small plugged tubes aseptically.

Duplicate tubes were inoculated, and one of each pair was covered with a 2 cm layer of sterile melted petroleum jelly. They were incubated at 25° for 14 days. Acid tubes become yellow, alkaline tubes become blue. The surface of the open tubes becomes blue if the carbohydrate is not attacked. "Oxidizers" turn only the top of the open tubes yellow; the depth remains green.

Strain	Glucose	Fructose	Mannose	Arabinose	Xylose	Salicin	Mannitol	Sorbitol	Sucrose	Maltose
MESOPHILS (C. violaceum)										
FH	F	F	F	-	-	-	-	F	-	?
MK	F	F	F	-	-	-	-	F	-	?
BH	F	F	F	-	-	-	-	F	F	?
BN	F	F	F	-	-	-	-	?	-	?
AM	F	F	F	-	-	-	-	F	-	?
MW	F	F	F	-	-	-	-	F	-	?
TV	F	F	F	-	-	-	-	F	-	?
LG	F	F	F	-	-	-	-	F	F	?
SL	F	F	F	-	-	-	-	F	F	?
RV	F	F	F	-	-	-	-	F	F	?
DK	F	F	F	-	-	-	-	?	-	?
TA	F	F	F	-	-	-	-	F	-	?
TB	F	F	F	-	-	-	-	F	-	?
PT	F	F	F	-	-	-	-	F	-	?
BM	F	F	F	-	-	NT	-	NT	-	NT
ATYPICAL MESOPHILS (C. violaceum)										
LW	O	O	-	-	-	-	-	?	-	?
SH	O	O	-	-	-	-	-	?	-	-
RT	O	O	O	-	-	-	-	?	-	-
MH	O	O	O1	-	-	-	-	?	-	-
PSYCHROPHILS (C. lividum)										
NT	O	O	O	O	O	-	O	?	-	O1
EA	O	O	O	O	O	?	O1	O1	-	O
EB	O	O	O	O	O	?	O	O	-	O
EC	O	O	O	O	O	?	O	O	O1	O
GA	O	O	O	O	O	-	O	O	O	O
DA	O	O	O	O	O	-	O1	O1	O	O
NC	O	O	O	O	O	?	O	O	O	O
MA	O	O	O	O	O	-	O	O	O	O
MB	O	O	O	O	O	?	O	O	O	O
MC	O	O	O	O	O	-	O	O	O	O
HA	O	O	O	O	O	-	O	O	O	O
HB	O	O	O	O	O	-	O	O	O	O
HC	O	O	O	O	O	-	O	O	O	O
HD	O	O	O	O	O	O1	O	O1	O	O
HE	O	O	O	O	O	?	O	O	O	O
HF	O	O	O	O	O	?	O	O1	O	O
IN	O	O	O	O	O	?	O	O	O	O

Strain	Glucose	Fructose	Mannose	Arabinose	Xylose	Salicin	Mannitol	Sorbitol	Sucrose	Maltose
Psychrophils (cont.)										
CA	O	O	O	O	O	Ol	O	O	O	O
PB	O	O	O	O	O	?	O	O	O	O
RU	O	O	O	O	O	?	O	O	O	O
ED	O	O	O	O	O	NT	O	NT	O	NT
EF	-	-	-	-	-	NT	-	NT	-	NT
BC	O	O	O	O	O	NT	-	NT	-	NT
OTHER BACTERIA										
GR	F1	O	O	-	-	-	-	O	O	O
TE	-	-	-	-	-	-	-	-	-	-
TI	O	O	O	O	-	Ol	Ol	Ol	-	O
NCTC 1377	F	F	F	F1	F1	NT	NT	NT	NT	F
NCTC 9493	F	F	F	F	F	NT	NT	NT	NT	F
NCTC 2000	O	O	O	NT	NT	NT	NT	NT	NT	NT
NCTC 6749	O	O	O	NT	NT	NT	NT	NT	NT	NT

Carbohydrate

Strain	Trehalose	Glycerol	Galactose	Starch	Lactose	m-Inositol	Cellobiose	Inulin	Dulcitol
MESOPHILS (C. violaceum)									
FH	F	-	-	-	-	-	-	-	-
MK	F	ol	-	F1	-	-	-	-	-
BH	F	-	-	-	-	-	-	-	-
BN	F	-	-	F	-	-	-	-	-
AM	F	ol	-	-	-	-	-	-	-
MW	F	ol	-	-	-	-	-	-	-
TV	F	-	-	-	-	-	-	-	-
LG	F	-	-	F	-	-	-	-	-
SL	F	-	-	-	-	-	-	-	-
RV	F	Ol	-	-	-	-	-	-	-
DK	F	-	-	-	-	-	-	-	-
TA	F	-	-	-	-	-	-	-	-
TB	F	-	-	-	-	-	-	-	-
PT	F	o	-	F1	-	-	-	-	-
BM	F	NT	NT	-	-	NT	NT	NT	NT

Strain	Trehalose	Glycerol	Galactose	Starch	Lactose	m-Inositol	Cellobiose	Inulin	Dulcitol
ATYPICAL MESOPHILS (C. violaceum)									
LW	O	-	-	-	-	-	-	-	-
SH	O	-	-	-	-	-	-	-	-
RT	O	-	-	-	-	-	-	-	-
MH	O	O	-	-	-	-	-	-	-
PSYCHROPHILS (C. lividum)									
NT	-	-	Ol	-	O	o	Ol	-	-
EA	-	Ol	O	-	ol	o	-	-	-
EB	-	O	O	-	Ol	O	-	-	-
EC	-	-	-	-	Ol	O	-	-	-
GA	-	-	O	-	Ol	O	-	-	-
DA	-	-	O	-	O	O	-	-	-
NC	-	O	O	-	Ol	O	O	Ol	-
MA	-	O	O	-	Ol	O	-	O	-
MB	-	O	O	-	Ol	O	-	O	-
MC	-	O	O	-	O	O	O	O	-
HA	-	O	O	-	Ol	O	O	-	-
HB	-	O	O	-	Ol	O	-	-	-
HC	-	O	O	-	Ol	O	O	-	-
HE	-	O	O	-	Ol	O	Ol	-	-
HF	-	O	O	-	Ol	O	O	O	-
IN	-	O	O	-	Ol	O	O	O	-
CA	-	O	O	-	Ol	O	O	-	-
PB	-	O	O	-	Ol	O	O	O	-
RU	-	O	O	-	Ol	O	-	O	-
ED	?	NT	NT	-	Ol	NT	NT	NT	NT
EF	-	NT	NT	-	-	NT	NT	NT	NT
BC	-	NT	NT	-	Ol	NT	NT	NT	NT
OTHER BACTERIA									
GR	O	-	O	-	O	-	O	-	NT
TE	-	-	-	-	-	-	-	-	-
TI	Ol	-	O	-	O	O	O	-	NT
NCTC 1377	F	F	F	F	-	F	F1	NT	NT
NCTC 9493	F	F	F	F1	F	F	F	NT	NT
NCTC 2000	-	O	O	-	-	-	-	NT	NT
NCTC 6749	ol	O	O	-	-	-	-	NT	NT

Symbols: F = Fermentative attack (acid anaerobically); O = Oxidative attack only (acid only aerobically); o = slight acid aerobically; l = late reaction in 7 or more days; - = no acidity; ? = doubtful acidity; NT = not tested.

Protocol 10. Reactions in litmus milk

The medium was prepared by the method of Mackie and McCartney (1948, p.163), inoculated and incubated at 25°.

	3 days	14 days		3 days	14 days
	MESOPHILS (C. violaceum)			PSYCHROPHILS (C. lividum)	
FH	N - - -	alk, c, p, -	NT	N, - - -	ac, C, p, -
MK	N C p -	Alk, c, p, -	EA	N, - - -	ac, C, - b
BH	ac - - -	alk, c, P, -	EB	N, - - -	Alk, C, - -
BN	N c - -	alk, c, P, -	EC	alk, - - -	Alk, - - -
AM	N - p -	ac, c, P, -	GA	N, - - -	Alk, C, - B
MW	N - - -	ac, c - -	DA	N, - - -	Alk, C, - -
TV	N - - -	ac, c, p, -	NC	N, - - -	Alk, C, - -
LG	N - - -	ac, c, P, -	MA	N, - - -	Alk, C, - B
SL	N - - -	ac, C, P, -	MB	N, - - -	alk, C, - b
RV	N - - -	N, c, p, -	MC	N, - - -	Alk, C, - B
DK	ac, c, p, -	alk, C, P, -	HA	alk, - - -	Alk, C, - -
TA	N - - -	N, c, P, -	HB	alk, - - -	Alk, C, - B
TB	ac, - - -	N, c, p, -	HC	Alk, - - -	Alk, - - -
PT	N, - - -	Alk, C, p, -	HD	alc, - - -	Alk, - - -
LW	N, - - -	ac, C, p, -	HE	Alk, - - b	Alk, c, p, B
SH	ac - - -	ac, C, p. -	HF	alc, - - -	Alk, C, - B
RT	alk, - - -	ac, C, p, -	IN	N, - - B	Alk, C, - B
MH	alk, - - -	N, c, - -	CA	alk, c, - -	Alk, C, - -
			PB	alc, - - -	Alk, C, - b
			RU	N, - - -	N, c, p, b

OTHER BACTERIA

Chromobacterium viscosum		GR	N, - - -	ac, c, p. B
Chromobacterium iodinum		TE	N, - - -	Alk, c, - -
Chromobacterium ianthinum		TI	N, - - -	ac, c, - -
Serratia marcescens	NCTC 1377		Ac, C, - -	Ac, c, P, B
Serratia marcescens	NCTC 9493		Ac, C, - -	Ac, c, P, B
Pseudomonas aeruginosa	NCTC 2000		N, - - b	ac, c, p, B
Pseudomonas aeruginosa	NCTC 6749		N, - - -	ac, c, P, B

Symbols: The features are scored in the order - reaction, clot, peptonization, bleaching.
Ac = acid, Alk = alkaline, N = neutral,
C = clot, P = peptonization, B = bleaching
- = no change.
Small case letters indicate a slight degree of the feature.

Protocol 11. Biochemical tests: 1.

Indole production. Peptone water cultures, incubated for 5 days at 25° were tested with ether and the Böhme indole reagents as given in Wilson and Miles (1946, Vol. 1, p. 367). A red colour indicates indole.

Ammonia production. Peptone water cultures incubated at 25° for 5 days were tested by adding Nessler's reagent (Wilson and Miles, 1946, Vol. 1, p. 369). A brown colour indicates ammonia.

Methyl red and Voges-Proskauer tests. Triplicate cultures in glucose phosphate peptone medium (Wilson and Miles, 1946, Vol. 1, p. 368) incubated for 5 days at 25° were used. To one was added methyl red solution (red = positive reaction), to the second was added creatinine and caustic potash (the O'Meara test - red = positive V-P) and to the third α-naphthol and caustic potash (Barritt's method - red = positive V-P) according to the techniques given in Wilson and Miles (1946, Vol. 1, p. 368).

H_2S production. Lead acetate paper was placed between the plug and wall of a tube of nutrient broth culture and observed for blackening for 7 days at 25°.

Methylene blue reduction. To a 5 ml nutrient broth culture, grown at 25° for 24 hours, was added one drop of 1% aqueous methylene blue and it was incubated for one hour at 25°. Complete decolorization was a strongly positive reaction, a green colour was a weakly positive reaction.

Reduction of nitrate to nitrite. Duplicate cultures in nutrient broth containing 0.1% of potassium nitrate and 0.25% of agar were incubated at 25°. One culture was tested after 24 hours, and the other after 5 days. The test consisted of adding 1 ml of a 1% solution of sulfanilic acid in 25% acetic acid, followed by 1 ml of a 0.5% solution of dimethyl α-naphthylamine in 25% acetic acid. A red colour indicated nitrites. All negative reactions were checked by adding zinc dust to see whether any unchanged nitrate remained (ZoBell, 1932); if no red colour appeared it showed that no nitrate remained, and the organism had in fact reduced all the nitrate and also had destroyed all the nitrite formed from the nitrate.

Destruction of nitrite. Duplicate cultures were made in nutrient broth containing 0.25% of agar and 0.0005% (i.e. 5 μg/ml) of sodium nitrite. They were incubated at 25°, one tube being tested after one day, and the other after 5 days. With nonmotile organisms, the tubes were well shaken after one day, since the bacteria do not migrate throughout the medium. The test was done as in the previous test. Absence of a pink colour showed that the nitrite had been destroyed.

Catalase. A 48 hour culture grown at 25° on nutrient agar was used. Two methods were used—hydrogen peroxide ("20 volumes per cent") was poured onto the slope and it was examined for bubbles, or a loopful of a growth was emulsified in a drop of the hydrogen peroxide and examined for bubbles. The latter method was more sensitive.

Results

Strain	Indole	Ammonia	Methyl red	Voges-Proskauer	H_2S	Reduction of methylene blue	Reduction of nitrate to nitrite	Destruction of nitrite	Catalase (slide test)
				MESOPHILS (C. violaceum)					
FH	-	+++	?	-	-	+	-	-	+
MK	-	+++	-	-	-	±	+	+	++
BH	-	+++	?	-	-	+	+	+	++
BN	-	++	?	-	-	+	+	+	++
AM	-	+	?	-	?	±	+	±	+
MW	-	+++	?	-	?	+	+	+	+
TV	-	+++	?	-	-	+	+	++	++
LG	-	+++	?	-	?	+	+	+	++
SL	-	+	?	-	?	+	+	-	+
RV	-	+	?	-	-	±	+	+	+
DK	-	+	?	-	?	+	+	+	+
TA	-	+++	?	-	-	+	+	++	+
TB	-	+++	?	-	-	+	+	+	++
PT	-	++	-	-	-	±	+	+	++
LW	-	+	-	-	?	±	+	±	+
SH	-	+	-	-	-	±	+	±	+
RT	-	+	?	-	?	±	+	-	++
MH	-	++	-	-	-	+	+	+	+
BM	-	+++	NT	NT	NT	NT	+	NT	+
				PSYCHROPHILS (C. lividum)					
NT	-	+++	-	-	-	-	-	-	-
EA	-	±	-	-	-	±	+	++	±
EB	-	++	-	-	-	?	+	±	+
EC	-	±	-	-	-	?	+	+	+
GA	-	+	-	-	-	?	+	±	+
DA	-	++	-	-	-	?	+	-	++
NC	-	+	-	-	-	?	+	-	+
MA	-	++	-	-	-	?	+	±	+
MB	-	+	?	-	-	-	-	+	+
MC	-	+++	?	-	-	-	+	++	±
HA	-	++	-	-	-	?	+	+	+
HB	-	+++	-	-	-	?	+	+	+
HC	-	+++	-	-	-	?	+	+	+
HD	-	+++	-	-	-	±	+	+	+
HE	-	++	-	-	-	±	+	+	+
HF	-	++	-	-	-	±	+	+	+
IN	-	++	-	-	-	-	+	+	±
CA	-	+++	-	-	-	±	+	+	+

Strain	Indole	Ammonia	Methyl-red	Voges-Proskauer	H_2S	Reduction of methylene blue	Reduction of nitrate to nitrite	Destruction of nitrite	Catalase (slide test)
				Psychrophils (cont.)					
PB	-	+++	-	-	-	±	+	+	+
RU	-	++	-	-	-	-	+	+	+
ED	-	++	NT	NT	NT	NT	+	NT	+
EF	-	++	NT	NT	NT	NT	+	NT	±
BC	-	++	NT	NT	NT	NT	+	NT	+
				OTHER BACTERIA					
GR	-	-	-	-	-	-	+	±	+
TE	-	-	-	-	-	±	-	-	+
TI	-	++	-	-	-	±	-	-	+
NCTC 1377	?	+++	-	?	+++	+	+	+	+
NCTC 9493	?	++	-	?	+++	+	+	+	+
NCTC 2000	-	+++	-	-	-	±	+	+	++
NCTC 6749	-	+++	-	-	-	±	+	+	++

Symbols: + = definite positive; - = negative; NT = not tested;
? = doubtful result.
Ammonia and catalase tests: ± to +++ = increasing degrees from just detectably positive to strongly positive.
Methylene blue reduction: + = fluid colourless or almost so; ± = fluid green; - = no change.
Nitrite destruction: ± = faint pink after 5 days growth;
+ = colourless after 5 days growth;
++ = colourless after 1 days growth.

Protocol 12. Biochemical tests: 2

Urease. Urea agar slopes (Christensen, 1946) were inoculated and incubated at 25° for 4 days.

Phosphatase. The method used was that of Bray and King (1943) the medium being inspissated egg plates containing 0.4% of calcium phenolphthalein diphosphate and 20% of broth. Plates were streaked with a loopful of a young broth culture and tested after 2 days growth at 25°, by inverting the plates over a dish of strong ammonia. A red colour indicated phosphatase.

Hydrolysis of aesculin. The medium used was based on that of Harrison and van der Leck (1909) in which iron is the indicator of hydrolysis of aesculin. It consisted of 1% peptone, 0.1% aesculin and 0.05% of ferric citrate (scales) in distilled water. It was used in tubes, and also plates of the medium solidified with 1.5% of agar were made and the tests put up in parallel. Both were sterilized by autoclaving at 115° for 15 minutes. Tubes and plates were inoculated with a loopful of young broth culture and incubated at 25°. The tubes were watched for 14 days and the plates for 4 days. A positive result is shown by a black colour in the liquids, or a blue-black halo around the colonies on the plates.

Results

Strain	Urease (7 days)	Phosphatase (2 days)	Aesculin hydrolysis	
			Tubes (14 days)	Plates (2 days)
MESOPHILS (C. violaceum)				
FH	-	+++	-	-
MK	-	+++	-	-
BH	-	+	-	-
BN	-	+	-	-
AM	-	+++	-	-
MW	-	++	-	-
TV	-	+++	-	-
LG	-	++	-	-
SL	-	++	-	-
RV	-	+++	-	-
DK	-	+++	-	-
TA	-	+++	-	-
TB	-	++	-	-
PT	-	+++	-	-
LW	-	+++	-	-
SH	-	++	-	-
RT	-	+++	-	-
MH	-	+	?	-
BM	NT	NT	-	-
PSYCHROPHILS (C. lividum)				
NT	-	++	+++	++
EA	-	++	+++	++
EB	-	+++	+++	++
EC	-	+++	+++	++
GA	-	+++	+++	+++
DA	-	++	+++	+++
NC	-	++	++	+++
MA	-	+++	++	+++
MB	-	++	+++	+++
MC	-	+++	+++	++
HA	-	+++	+++	+++
HB	-	++	+++	+++
HC	-	+++	+++	+++
HD	-	+++	+++	+++
HE	-	+++	+++	+++
HF	-	+++	+++	+++
IN	-	+++	++	+++
CA	-	++	++	+++
PB	-	+++	+++	+++
RU	-	+++	++	+++
ED	NT	NT	-	-
EF	NT	NT	-	-
BC	NT	NT	+++	+++

OTHER BACTERIA

Strain	Urease (7 days)	Phosphatase (2 days)	Aesculin hydrolysis Tubes (14 days)	Aesculin hydrolysis Plates (2 days)
GR	-	++	+	±
TE	-	++	-	-
TI	-	++	+++	+++
NCTC 1377	-	+	+++	+++
NCTC 9493	-	?	+++	+++
NCTC 2000	-	+	-	-
NCTC 6749	-	++	++	++

Symbols: Phosphatase and aesculin plates: ± to +++ = increasing degrees of reaction from colour changed only in the growth to wide zone around growth.
Aesculin tubes: ± to +++ = increasing degrees of blackening from just detectable to inky black.

Protocol 13. Biochemical tests: 3

Haemolysis. 5% horse blood was added to nutrient agar and plates were inoculated with a loopful of young broth culture and incubated at 25° for 2 days and 4 days.

Hydrolysis of casein, gelatin and starch. The methods were those of Smith, Gordon and Clark (1952, pp. 41, 43), which were found very convenient. The details are as follows:

Casein hydrolysis. Plates composed of equal parts of sterile skim milk and of watery 3% agar were inoculated with a loopful of young broth culture and observed for zones of clearing when incubated at 25° for 2 and 4 days.

Gelatin hydrolysis. Plates of nutrient agar containing 0.4% of gelatin were inoculated and incubated at 25° for 4 days. They were then flooded with the following solution: mercuric chloride 15 g, concentrated hydrochloric acid 20 ml, water 100 ml, and examined for zones of clearing and (after scraping off the growth) for clearing below the growth.

Starch hydrolysis. Plates of nutrient ager containing 1% of soluble starch were inoculated and after incubation at 25° for 4 days, were flooded with Lugol's iodine and examined for colourless zones around and under the growth (after scraping the growth away).

Egg yolk reaction. Egg yolk plates (Knight and Proom, 1950) consisting of nine parts of nutrient agar and one part of a Seitz-filtered mixture of one egg-yolk in 250 ml of physiological saline (Macfarlane, Oakley and Anderson, 1941) were inoculated with a loopful of young broth culture,

and watched for opaque zones around the growth during incubation for 4 days at 25°. A strain of Bacillus cereus was used as a positive control.

Digestion of Löffler's serum. Slopes (see Protocol 3) were incubated by streaking and incubated at 25° for 14 days.

Results

Strain	Hydrolysis of casein (2 days)	Hydrolysis of casein (4 days)	Hydrolysis of gelatin (4 days)	Hydrolysis of starch (4 days)	Haemolysis on horse blood agar (2 days)	Haemolysis on horse blood agar (4 days)	Egg yolk reaction (2 days)	Egg yolk reaction (4 days)	Löffler's serum digestion (14 days)
MESOPHILS (C. violaceum)									
FH	++	+++	++	-	+	+++	+	++	++
MK	++	+++	+++	-	++	+++	++	+++	++
BH	++	+++	+++	-	++	+++	++	+++	++
BN	++	+++	+++	-	++	+++	++	+++	++
AM	+	++	+++	-	-	+++	++	+++	++
MW	++	+++	+++	-	++	+++	++	+++	++
TV	++	+++	+++	-	++	+++	++	+++	++
LG	++	+++	+++	±	++	+++	++	+++	++
SL	++	+++	+++	-	++	+++	++	+++	++
RV	++	+++	+++	-	++	+++	++	+++	++
DK	++	+++	+++	-	++	+++	++	+++	++
TA	++	+++	+++	-	++	+++	++	+++	++
TB	++	+++	+++	-	++	+++	++	+++	++
PT	++	+++	+++	±	±	+++	+	++	+
LW	++	+++	++	-	±	+++	+	++	++
SH	++	+++	++	-	±	+	+	++	++
RT	+	+++	++	-	±	+++	++	+++	++
MH	++	+++	++	-	+	+++	++	+++	++
BM	++	+++	++	-	++	+++	++	+++	++
PSYCHROPHILS (C. lividum)									
NT	+	+	-	-	-	-	-	-	±
EA	-	±	±	-	±	+	-	-	-
EB	-	-	±	-	-	-	-	-	+
EC	-	-	-	-	-	-	-	-	-
GA	-	±	-	-	-	-	-	-	-
DA	-	+	±	-	-	-	-	-	-
NC	-	-	-	-	-	-	-	-	±
MA	-	-	-	-	-	+	-	-	-
MB	±	±	±	-	-	+	-	-	±
MC	+	+++	±	-	-	+	-	-	-
HA	±	+	±	-	-	-	-	-	-
HB	-	±	-	-	-	-	-	-	-
HC	-	±	-	-	-	-	-	-	-
HD	-	±	-	-	-	-	-	-	+
HE	±	++	-	-	±	+	-	-	-
HF	-	±	±	-	-	-	-	-	+
IN	-	-	-	-	-	-	-	-	-
CA	-	±	-	-	-	-	-	-	-
PB	-	-	-	-	-	-	-	-	-
RU	±	+	-	-	-	-	-	-	-
ED	±	+	-	-	-	-	-	-	-
EF	-	-	-	-	-	-	-	-	-
BC	±	+	-	-	-	±	-	-	-

Strain	Hydrolysis of casein (2 days)	Hydrolysis of casein (4 days)	Hydrolysis of gelatin (4 days)	Hydrolysis of starch (4 days)	Haemolysis on horse blood agar (2 days)	Haemolysis on horse blood agar (4 days)	Egg yolk reaction (2 days)	Egg yolk reaction (4 days)	Löffler's serum digestion (14 days)
OTHER BACTERIA									
GR	-	+	+++	-	-	±	-	-	-
TE	-	-	+++	-	++	+++	-	-	-
TI	±	++	+++	-	±	++	+	++	+
NCTC 1377	+++	+++	+++	+	+	+++	++	+++	++
NCTC 9493	+++	+++	+++	-	++	+++	++	+++	++
NCTC 2000	+	+++	+++	-	±	++	?	+	++
NCTC 6749	+++	+++	+++	-	±	++	?	+	++

Symbols. ± to +++ = increasing zones of reaction from barely visible (restricted to beneath the growth) to about 5 mm in width. Löffler's serum ± to ++ increasing degrees of digestion from slight softening to collapse of the medium.

Protocol 14. Biochemical tests: 4

Utilization of malonate and production of phenylpyruvic acid. This was tested in small tubes containing 3 ml of the convenient combined medium of Shaw and Clarke (1955), after growth at 25° for 2 days. The medium consists of NaCl 2.0 g, KH_2PO_4 0.4 g, K_2HPO_4 0.6 g, $(NH_4)_2SO_4$ 2.0 g, sodium malonate 3.0 g, DL-phenylalanine 2.0 g, "Yeastrel" (Brewer's Food Supply Co., Ltd., Edinburgh) 1.0 g, 0.5% ethanolic bromthymol blue 5 ml., distilled water to 1 litre, and is sterilized by autoclaving. If malonate was utilized for growth, the medium became blue. On adding 0.1 ml of 0.1 N-HCl and then 0.1 ml of 0.5 M-ferric chloride, a green colour showed the presence of phenyl-pyruvic acid (which was checked with a strain of Proteus OX 19).

Gluconate test. This was tested by growing the strains in 3 ml of the modified Hayne's medium of Shaw and Clarke (1955) in tubes of 22 mm internal diameter, for 4 days at 25°. The medium consists of peptone 1.5 g, "Yeastrel" 1.0 g, K_2HPO_4, 1.0 g, potassium D-gluconate 40 g, distilled water to 1 litre. The pH is adjusted to 7.0 and it is sterilized by autoclaving at 115° for 10 minutes. After 4 days, 1 ml of Benedict's Qualitative Reagent for Glucose was added, and the tubes kept at room temperature for 10 minutes and examined. They were then heated in a boiling water bath for 10 minutes. A yellow or orange precipitate showed the presence of 2-keto-gluconic acid or other reducing substances. Some cultures gave reduction in the cold. 2-keto-D-gluconic acid only reduces Benedict's solution on heating, but ascorbic acid will do so in the cold.

Arylsulfatase. Cultures in peptone water containing 0.001 M-potassium phenolphthalein disulfate (Whitehead, Morrison and Young, 1952) were incubated for 7 days at 25°, and the N-NaOH was added drop by drop. A red colour indicated hydrolysis. The positive control was medium after boiling with an equal volume of concentrated hydrochloric acid.

Results

Strain	Utilization of malonate	Production of phenylpyruvate	Gluconate	Arylsulfatase	Strain	Utilization of malonate	Production of phenylpyruvate	Gluconate	Arylsulfatase
	MESOPHILS (C. violaceum)					PSYCHROPHILS (C. lividum)			
FH	-	-	-	-	NT	-	-	±	-
MK	-	-	-	-	EA	-	-	+++	-
BH	-	-	-	-	EB	-	-	+++	-
BN	-	-	-	-	EC	-	-	+	-
AM	-	-	-	-	GA	-	-	-	-
MW	-	-	±	-	DA	-	-	-	-
TV	-	-	-	-	NC	-	-	-	-
LG	-	-	-	-	MA	-	-	-	-
SL	-	-	-	-	MB	-	-	-	-
RV	-	-	-	-	MC	-	-	-	-
DK	-	-	-	-	HA	-	-	+++	-
TA	-	-	-	-	HB	-	-	+++	-
TB	-	-	-	-	HC	-	-	++	-
PT	-	-	-	-	HD	-	-	-	-
LW	-	-	-	-	HE	-	-	++	-
SH	-	-	-	-	HF	-	-	-	-
RT	-	-	-	-	IN	-	-	-	-
MH	-	-	-	-	CA	+	-	-	-
					PB	-	-	-	-
					RU	-	-	-	-
					OTHER BACTERIA				
					GR	-	-	-	-
					TE	-	-	-	-
					TI	-	-	+++	-
					NCTC 1377	-	-	+++	-
					NCTC 9493	-	-	+++	-
					NCTC 2000	?	?	+++H	-
					NCTC 6749	+	?	+++H	-

Symbols. Malonate: + = medium blue; ? = doubtful.
Gluconate: ± to +++ increasing degrees of reduction of Benedict's, from a green colour to a heavy orange precipitate.
H = reduction only on heating.
? = doubtful result.

Protocol 15. Production of gas from nitrate.

The medium was that of ZoBell (1932) consisting of Bactopeptone (Difco) 2.0 g, beef extract (Lemco) 1.0 g, sodium chloride 1.0 g, potassium nitrate 1.0 g, agar 3.0 g, water 1 litre, with reaction adjusted to pH 6.8. It was placed in narrow tubes to a depth of 5 cm and autoclaved, and inoculated by stabbing. The tubes were incubated at 25° for 14 days and any gas bubbles recorded. The results are shown below.

Strain	Days of incubation 1	2	4	8	14	Strain	Days of incubation 1	2	4	8	14
	MESOPHILS (C. violaceum)						PSYCHROPHILS (C. lividum)				
FH	-	-	-	-	-	NT	-	-	g	-	-
MK	-	-	-	-	-	EA	-	-	-	-	-
BH	-	-	-	-	-	EB	-	-	-	-	-
BN	-	-	-	-	-	EC	-	-	-	-	-
AM	-	-	-	-	-	GA	-	-	-	G	G
MW	-	-	-	-	-	DA	-	-	-	g	G
TV	-	-	-	-	-	NC	-	-	g	G	G
LG	-	-	-	-	-	MA	-	-	-	g	G
SL	-	-	-	-	-	MB	-	-	g	G	G
RV	-	-	-	-	-	MC	-	-	-	-	-
DK	-	-	-	-	-	HA	-	-	-	-	-
TA	-	-	-	-	-	HB	-	-	-	-	-
TB	-	-	-	-	-	HC	-	-	-	-	-
PT	-	-	-	-	-	HD	-	-	-	-	-
LW	-	-	-	-	-	HE	-	-	-	-	-
SH	-	-	-	-	-	HF	-	g	G	G	G
RT	-	-	-	-	-	IN	-	-	-	-	-
MH	-	-	-	-	-	CA	-	-	-	-	-
						PB	-	-	-	-	-
						RU	-	-	g	G	G

Symbols. g = a few bubbles of gas.
G = many bubbles of gas.

Protocol 16. Digestion of chitin.

Colloidal chitin was prepared by the method of Tracey (1955) as follows: "cuttlefish bones" were soaked in 20% commercial hydrochloric acid for 24 hours to remove calcareous matter. The chitinous sheets were removed, dried by blotting, and suspended in cold concentrated hydrochloric acid (Sp. Gr. 1.18) for 8 hours, when most of it had dissolved. The solution was filtered through glass wool, and poured into ten times its volume of water, with stirring. After standing overnight the precipitate of chitin was separated and washed three times with distilled water by centrifugation, dialyzed overnight against running distilled water, and preserved under toluene. The chitin content was estimated

by evaporating an aliquot to dryness and weighing, and sufficient of the suspension was added to melted nutrient agar to give a final concentration of 0.5 mg/ml. Plates of this medium were inoculated in streaks, incubated at 25° and observed for zones of clearing.

Results

Strain	Days of incubation 4	7	14	Strain	Days of incubation 4	7	14
	MESOPHILS (C. violaceum)				PSYCHROPHILS (C. lividum)		
FH	-	-	-	NT	-	-	-
MK	+	++	+++	EA	-	-	-
BH	+	++	+++	EB	-	-	-
BN	+	++	+++	EC	-	-	-
AM	+	++	+++	GA	-	-	-
MW	++	++	+++	DA	-	-	-
TV	+	++	+++	NC	-	-	-
LG	++	+++	+++	MA	-	-	-
SL	+	++	+++	MB	-	-	-
RV	-	±	+	MC	-	-	-
DK	±	+	++	HA	-	-	-
TA	++	+++	+++	HB	-	-	-
TB	±	++	+++	HC	-	-	-
PT	-	+	++	HD	-	-	-
LW	-	±	+	HE	-	-	-
SH	-	±	+	HF	-	-	-
RT	-	±	+	IN	-	-	-
MH	±	±	+	CA	-	-	-
BM	-	+	++	PB	-	-	-
				RU	-	-	-
				ED	-	-	-
				EF	-	-	-
				BC	-	±	+

Symbols. - = no clearing; ± = clearing beneath growth; + to +++ = zones of clearing from c 1 mm to c 5 mm in width.

Protocol 17. Serological methods

Antisera. Antisera were prepared in rabbits by intravenous injection of suspensions of whole organisms. To make 'O' antisera the organisms were grown on nutrient agar for 24 hours at 30° for mesophils or 48 hours at 25° for psychrophils, and washed off the medium. The suspensions were heated at 100° for 2 hours and preserved with 0.1% of phenol. To make 'OH' antisera, nutrient broth cultures grown at 30° for 24 hours for mesophils or at 25° for 48 hours for psychrophils, were killed by adding formalin to give a concentration of 0.1% of formalin (0.04% of formaldehyde).

The rabbits were injected three times a week until they had received six injections in doses increasing from c. 10^7 to c. 5×10^8 organisms. They were bled ten days after the last injection. A few rabbits required further immunization to give good antisera. The sera were either stored at -20°, or, after mixing with an equal volume of glycerol, at 4°. No fall in titre has been noted after 5 years storage by either method.

Agglutinable suspensions. It was observed that treatment by heat or with alcohol often increased the autoagglutinability of suspensions, and, as shown below, the specificity broadens. Therefore, living suspensions consisting of cultures grown in nutrient broth (24 hours at 30° for mesophils, 48 hours at 25° for psychrophils) were used for O agglutination, and diluted in 0.17% sodium chloride (this low concentration reduced the autoagglutination of rough strains) until they had an opacity of 0.55 times that of the International Opacity standard (World Health Organization, 1954) i.e. 5.5 Opacity Units which is approximately equivalent to an optical density of 0.33 in a 1 cm cell with an Ilford H 508 neutral grey filter. For H agglutination cultures were treated with formalin (final concentration 0.04% of formaldehyde) and similarly diluted to 5.5 Opacity Units. Polar H suspensions were formolized broth cultures grown as for O suspensions. Peritrichous H suspensions were young cultures on nutrient agar (grown for 18 hours at 30°) washed off and formolized. The flagellation was confirmed by flagella staining. All suspensions were freshly prepared.

Agglutinations. Doubling dilutions of antiserum in 0.17% NaCl were made, usually starting at a dilution of 1 in 5, and 0.25 ml amounts were mixed in Dreyer tubes with 0.25 ml of bacterial suspension. For 'O' agglutination they were incubated at 56° for 4 hours and then at 20° overnight. For 'H' agglutination the incubation varied and is specified in each experiment. The titre was taken as the reciprocal of the final dilution of antiserum in the last tube showing agglutination visible to the naked eye.

Absorption of sera. When required (as for example in the experiment shown in Table 10 (Chapter XIV) sera were absorbed by mixing with an equal volume of a 20% packed suspension of organisms, and after 1 hour at 37° the organisms were removed by centrifugation. The absorbing suspensions were prepared as follows: O suspensions were nutrient broth cultures which were deflagellated by treatment for a few minutes in a Waring Blendor; polar H suspensions and peritrichous H suspensions were formolized suspensions of broth cultures or agar cultures prepared as described above under Agglutinable suspensions; they were concentrated by centrifugation. The adsorbed sera were titrated against the type of suspension used for absorption to confirm that absorption was complete, and if any agglutination was found at a dilution of 1 in 5 antiserum, they were absorbed again.

Effect of various treatments on the agglutinable suspensions. A few examples are given below. Living suspensions and formolized suspensions were prepared as described above. Alcoholized suspensions were organisms from living broth cultures mixed with 9 times their volume of 96% ethanol, and after standing at 20° for 1 hour, washed with 0.17% NaCl. "Boiled" suspensions were living broth cultures heated zt 100° for 30 minutes.

Antiserum	Suspension	Titre against suspensions of strains: MK	AM	TV	NT
MK 'O' serum	Living	1280	0	0	20
AM 'O' serum	Living	320	1280	0	0
TV 'O' serum	Living	40	0	640	0
NT 'O' serum	Living	20	0	0	160
MK 'O' serum	Alcoholized	80	0	80	20
AM 'O' serum	Alcoholized	40	80	320	0
TV 'O' serum	Alcoholized	20	20	80	0
NT 'O' serum	Alcoholized	20	0	0	40
MK 'O' serum	Boiled	80	0	40	40
AM 'O' serum	Boiled	40	160	80	0
TV 'O' serum	Boiled	40	20	80	0
NT 'O' serum	Boiled	20	40	40	80

The effect of formolization on polar and peritrichous suspensions differs, as shown in the illustration below.

Antiserum	Suspension	Titre against suspensions of strains: DK	MK
DK 'O' serum	Living, polar	640	-
	Formalized, polar	640	-
	Living, peritrichous	320	-
	Formalized, peritrichous	80	-
MK 'O' serum	Living, polar	-	640
	Formalized, polar	-	640
	Living, peritrichous	-	1280
	Formalized, peritrichous	-	160

Symbols. 0 = less than 10. - = not tested.

Protocol 18. Sensitivity of Chromobacterium strains to bacteriophages of other bacteria.

A large collection of high-titre phage preparations was kindly provided by Dr. I.N. Ascheshov, in the form of mixtures of phages. The mixtures, and the phages they contained were as follows.

Mixture	Phages (Dr. Ascheshov's code numbers)
1. Staphylococcal phages	1, 2, 3, 4, 5a, 5b, 6, 8, 9, 10, 10D, 11r, 12, 13, 13D, 14, 16, 18, 19, 20, S3K, 21, 22, 23, 24, 25, 26, 27, 29, 30.
2. Streptococcal phages	3, 4, 6, 7, Entero 1B, Entero 2B, Entero 4, Entero 5/7.

Mixture	Phages (Dr. Ascheshov's code numbers)
3. Cholera phages	B, C, D, E, F, G, H, I, J, K, M, R.
4. Escherichia coli phages	T1, T2, T3, T5, T7, 24b, 49b, 50br, 53a, FCZ, 4, 5, 6b, 6c, 12.
5. Miscellaneous	Salmonella typhosa 1, Salmonella paratyphi 1, Salmonella schottmulleri 1, Salmonella poona 1, Salmonella poona 2, Shigella flexneri 2, Shigella flexneri 3, Shigella dysenteriae S13, Bacillus cereus 1, Bacillus megaterium 1, Bacillus subtilis CSC, Actinomyces 1, Mycobacterium 169, Mycobacterium 908, Proteus Lab 1/Lab 1, Proteus 4/9, Proteus 5/9, Proteus 6/9.
6. Salmonella phages	2, 3, 5, 6, 7, 8.
7. Pseudomonas aeruginosa phages	1, 2A, 2B.

These mixtures were spotted onto nutrient agar plates which had been heavily spread with young broth cultures of all the Chromobacterium strains which had been received up to that time. The plates were incubated at 25° and examined for lysis after 18 and 48 hours. The strains tested were FH, MK, BH, BN, AM, MW, TV, LG, SL, RV, DK, LW, SH, RT, MH, TA, TB, NT, EA, EB, EC, GA, DA, NC, MA, MB, MC.

No lysis was produced by any of the seven mixtures upon any of the twenty-seven strains.

Protocol 19. Virulence tests.

Tryptic meat digest broth cultures were grown at 37° for 18 hours (25° for 18 hours for strain NT), and tenfold dilutions were made in 0.03M potassium phosphate buffer of pH 7.6. Viable counts were estimated by plating drops of known volume onto nutrient agar containing 5% of peptic digest of sheep's blood (Fildes, 1920), and 0.1 ml quantities of the dilutions were injected intraperitoneally into white mice of 20 g weight, or 1 ml quantities intraperitoneally into guinea pigs of 250 g weight. Some strains were then passaged five times through guinea pigs (using massive inocula of 18-hour broth cultures of organisms from the heart blood) and then the LD_{50} was again estimated as above. The animals were observed for one week, when the death rate was scored. Some of the animals were examined post-mortem and cultures made from the organs.

I Virulence tests in mice.
Each dilution was injected into 10 mice

Strain	No. of viable organisms injected	Deaths	Strain	No. of viable organisms injected	Deaths
FH	7.8×10^5	0	MW	5.0×10^5	0
	7.8×10^6	1***		5.0×10^6	0
	7.8×10^7	1***		5.0×10^7	1***
MK	4.4×10^5	1*	TV	4.9×10^5	0
	4.4×10^6	8*		4.9×10^6	4*
	4.4×10^7	9		4.9×10^7	5
BH	3.7×10^5	1*	NT	1.1×10^5	1***
	3.7×10^6	6*		1.1×10^6	1***
	3.7×10^7	8		1.1×10^7	0
BN	3.6×10^5	2*	LG	7.1×10^5	1***
	3.6×10^6	7*		7.1×10^6	3**
	3.6×10^7	10		7.1×10^7	8**
BHR/1	5.9×10^5	0	SH	1.7×10^5	0
	5.9×10^6	2*		1.7×10^6	1***
	5.9×10^7	8		1.7×10^7	2***
BNR/1	5.3×10^5	2**	LW	1.2×10^5	0
	5.3×10^6	4		1.2×10^6	0
	5.3×10^7	9		1.2×10^7	0
AM	5.3×10^5	0	RT	1.6×10^5	0
	5.3×10^6	1***		1.6×10^6	1***
	5.3×10^7	***		1.6×10^7	1***

* Organism recovered from heart blood or spleen.
** Organism recovered from nodular lesions in liver.
*** Organism not recovered from heart blood, liver or spleen.

Large doses usually caused death from septicaemia within 24 hours; small doses often caused death after several days and necrotic areas were seen in liver, spleen or lungs.

11 Virulence tests in guinea pigs

Each dilution was injected into 5 guinea pigs.

Strain	BEFORE PASSAGE Number of viable organisms injected	Deaths	AFTER PASSAGE Number of viable organisms injected	Deaths
FH	4.6×10^7	1***	6.2×10^6	0
	4.6×10^8	0	6.2×10^7	1*
	4.6×10^9	5*	6.2×10^8	5
MK	2.3×10^8	4*	1.3×10^7	0
	2.3×10^9	3	1.3×10^8	2*
	2.3×10^{10}	5	1.3×10^9	5
BH	1.3×10^8	0	1.7×10^7	3**
	1.3×10^9	5*	1.7×10^8	4
	1.3×10^{10}	4	1.7×10^9	4
BN	2.1×10^8	4*	7.6×10^5	2*
	2.1×10^9	4	7.6×10^6	4
	2.1×10^{10}	4	7.6×10^7	5
BHR/1	1.2×10^8	0	7.6×10^6	0
	1.2×10^9	0	7.6×10^7	0
	1.2×10^{10}	4*	7.6×10^8	4
BNR/1	1.3×10^8	0	1.1×10^7	0
	1.3×10^9	4*	1.1×10^8	1***
	1.3×10^{10}	5	1.1×10^9	5
AM	4.9×10^5	0	Not tested	
	4.9×10^6	0		
	4.9×10^7	0		
MW	4.6×10^5	0	Not tested	
	4.6×10^6	0		
	4.6×10^7	0		
TV	6.9×10^5	0	Not tested	
	6.9×10^6	0		
	6.9×10^7	0		
LG	9.4×10^7	0	5.2×10^6	0
	9.4×10^8	3*	5.2×10^7	5*
	9.4×10^9	5	5.2×10^8	5
LW	1.0×10^5	0	Not tested	
	1.0×10^6	0		
	1.0×10^7	0		

Strain	BEFORE PASSAGE Number of viable organisms injected	Deaths	AFTER PASSAGE Number of viable organisms injected	Deaths
SH	5.0×10^7	0	5.2×10^6	0
	5.0×10^8	1***	5.2×10^7	2
	5.0×10^9	5	5.2×10^8	5
RT	7.4×10^7	0	1.3×10^7	0
	7.4×10^8	0	1.3×10^8	5
	7.4×10^9	4***	1.3×10^9	5
NT	7.0×10^4	0	Not tested	
	7.0×10^5	0		
	7.0×10^6	0		

* Organisms recovered from heart blood or spleen.
** Organisms recovered from nodular lesions in liver.
*** Organism not recovered from heart blood, liver, or spleen.

As with mice, large doses caused rapid septicaemic death and small doses often caused slower death with necrotic areas in liver, spleen, or lungs.

Protocol 20. Clinical summary of Case 1.
(No.11 in Sneath et al., 1953, No.14 in Table 14, Chapter XIX)

This patient was a male British soldier aged 20 who was admitted to British Military Hospital, Singapore, in 1952 with symptoms of a urinary infection. The urine gave on blood agar and MacConkey agar a pure growth of a mesophilic strain of Chromobacterium (strain BH, see Table 1, Chapter III). It was later realized that the organism was probably not a contaminant, but the case notes could not then be traced. It is believed that he recovered under the usual treatment for urinary infections.

Protocol 21. Clinical case notes of Case 2.
(No.12 in Sneath et al., 1953, No.15 in Table 14, Chapter XIX)

I am indebted to Major D. Edwards, R.A.M.C., for the following case notes, which have been slightly edited where necessary to clarity. Chest X-rays and the temperature chart are shown in Figs. 13 and 14.

British Army Officer, aged 25. Admitted to British Military Hospital, Kamunting, Taiping, Malaya on 16th February 1952.

History. Quite well until 9 days ago—first noticed an ulcer on the left thigh which he believed was due to the magazine of his Sten gun rubbing against his thigh, followed by enlargement of the glands in his left groin. Treated himself with Sulfamezathine 8 grams.

7 days ago—General malaise, swelling, Temperature 101°, during next few days felt hot and cold, sweating and temperature varied between 100-104°F. During this period he treated himself with Procaine Penicillin 300,000 units daily.

Day of admission—pain in right shoulder on inspiration.
No cough, no chest pain.
No diarrhoea, no vomiting.
No urinary symptoms.

Past history. No previous illnesses. Movements: Malaya 17 months, Tapah, Kuala Lumpur, Tapah and North Perak.

Condition on Admission. Temperature 101°. Looks ill, pale, sweating. Mucous membranes good colour. No conjunctival injection. Fundi normal.

Mouth: Fauces injected, tongue moist white furred.

Neck: No enlarged glands palpable. No neck stiffness.

Trachea central.

Chest: Moves equally, resonant. Movement diminished on right side. Breath sounds vesicular, no added sounds. Pulse 90/minute. Blood pressure 120/80. Heart, nothing abnormal discovered.

Abdomen: Slight tenderness on compression of right lower chest and at right costal margin. Area of liver dullness extends 1 inch below costal margin, no extension upwards. Liver edge not palpable. No visc palpable.

Axillae: Firm small glands both sides.

Groins: Firm enlarged lymph glands in left groin.

On left thigh there is a round shallow ulcer about 1" diameter with black crusted base.

Reflexes present, equal; Plantars downgoing.

White blood cells 14,000/cu.mm. Polymorphs 82%. Lymphocytes 15%. Monocytes 1%. Eosinophils 2%. Basophils 0%.

Blood slides: No malaria parasites.

Chest X-ray: Normal. Screening: Diminished movement of right diaphragm.

Stools: Semi-formed, no blood or mucus, scanty Trichomonas hominis and occasional leucocytes seen.

Diagnosis. Provisional diagnosis of amoebic hepatitis. Treated with emetine, 65 mg daily by injection and chloroquine 0.6 g daily by mouth.

18.2.52. Temperature 104°F. Complained of pain in right shoulder on inspiration. Liver dullness 2 inches below costal margin, tender on palpation. Upper border of liver dullness in 6th intercostal space in mid-clavicular line.

19.2.52. Still complains of pain in right shoulder. Temperature 103°F. Pulse 120/minute. Neck stiffness present. Liver dullness 3 inches below costal margin, tender, no extension upwards of area of liver dullness. Pleural rub at right base.

Lumbar puncture: C.S.F. pressure 120 mms of water. Clear colourless fluid. Cells less than 5/cu.mm. Protein 70 mgms%. Glucose 95 mgms%. Chlorides 690 mgms%.

White blood cells 14,500/cu.mm. Polymorphs 83%. Lymphocytes 11%. Monocytes 6%.

Urine: Albumin +, few granular casts. Chlorides 3 grams/litre.

Seen by Colonel Hughes, opinion—Amoebic Hepatitis, and suggested Aureomycin 1 g stat, 0.5 g 6 hourly and Emetine and Chloroquine to be continued.

20.2.52. Temperature 99°F, feeling much better. Pulse 110/min. Liver dullness 2 inches below costal margin.

Blood cultures: Staphylococcus albus only.

21.2.52. Temperature 101°. Pulse 110. No extension of liver dullness upwards, no pleural rub heard. Ulcer on left thigh clean, granulating base now healing.

22.2.52. Temperature 98.4°. Pulse 70. Liver dullness 1 inch below costal margin tenderness less marked.

24.2.52. Afebrile, no liver tenderness, liver dullness extends 1 inch below costal margin.

28.2.52. Feels well, eating well, area of liver dullness unchanged but no liver tenderness. Emetine, Chloroquine and Aureomycin stopped.

2.3.52. Feels very well, now getting up, physical signs unchanged. Afebrile.

3.3.52. Complained of occipatal and right parietal headache with neck stiffness.

On examination: Slight neck stiffness. Liver dullness 1 inch below costal margin, no extension upwards, no liver tenderness. Spleen not palpable. No enlarged lymph glands. No other abnormal signs.

White blood cells 10,800/cu.mm. Polymorpht 74%, Lymphocytes 22%, Monocytes 3%, Basophils 1%.

5.3.52. General malaise, no headache but complains of pain over right upper chest on inspiration.

Temperature 101°F. Neck stiffness, Kernig's sign +

No muscular tenderness, power of all movements good and equal.

Reflexes: Abdominal reflexes absent, triceps jerks and knee jerks absent, ankle jerks both sluggish, biceps jerks and supinator jerks absent on the left and sluggish on the right. Plantar reflexes both downgoing.

Chest: No abnormal signs.

Area of liver dullness 2 inches below costal margin, tender, firm. Slightly tender glands in left groin.

Chest X-ray: High "domed" right diaphragm.

Lumbar puncture—pressure 120 mms of C.S.F: Crystal clear fluid. No cells seen. Protein 40 mgms%. Chlorides 700 mgms.

Serum agglutination titres: Proteus: OXK Nil, OX2 1/25, OX19 1/25. Typhoid: 1/25. Brucellus abortus and melitensis - Nil.

Blood culture: No growth.

6.3.52. Temperature 102.4°. Neck stiffness still present. Liver dullness 2 inches below costal margin and tenderness in epigastrium more marked. BP 110/70. Stools: no amoebae seen; culture: no pathogenic bacteria.

Emetine 65 mg and Chloroquine 0.2 g three times a day restarted.

8.3.52. Feels weak. Neck stiffness still present. Complains of pain in epigastrium and in right shoulder on inspiration, frequent loose stools.

Liver edge palpable 3 inches below costal margin, marked tenderness. BP 110/70. Pulse 110, regular. No muscular tenderness. Tendon reflexes absent, muscular power fair and equal.

Electrocardiogram: (Standard Limb leads) Rate 120. Rythm: regular. T1 taller than R2, Q3 prominent, T3 inverted.

Sigmoidoscopy—instrument passed to Recto-Sigmoid junction, normal mucosa with a little mucus, no ulceration, no bleeding seen. Swabs of mucus taken—no entamoebae seen.

Seen by Colonel Hughes and Professor Ransom: aspiration of liver, no pus obtained.

9.3.52. Condition unchanged, T. 102°F. Liver palpable (3 inches) spleen not palpable.

11.3.52. Complained of pain in right lower chest and in epigastrium. Temperature 103°F. Area of liver dullness extends upwards to 4th left interspace anteriorly, area of dullness extends across to left lower chest. Liver edge palpable 3 inches below costal margin, left lobe of liver palpable 3 inches below left costal margin, liver edge tender. White blood cells 15,400 per cu. mm. Polymorphs 85%, Lymphocytes 9%, Monocytes 6%.

12.3.52. General condition unchanged; physical signs in status quo ante except that there is now a pleural rub at the right base.

Portable Chest X-ray: Doming of right diaphragm, lung fields clear. (Fig.13).

13.3.52. Respirations 40 per minute, is very ill, pale, sweating, pulse 140 per minute. BP 110/70.

Aspiration of liver—40 ccs of greenish pus obtained from right lobe.

Physical signs unchanged.

Culture of pus—Chromobacterium violaceum.

Died at 10.20 p.m.

Protocol 22. Post-mortem examination of Case 2.
(No.12 in Sneath et al., 1953, No.15 in Table 14, Chapter XIX)

Post-mortem examination by Major P.H.A. Sneath, R.A.M.C., on 14th March, 1952. Date of death, 13th March 1952.

External Examination.

The body is that of a well built young man of about 25 years of age. There is moderate emaciation. Rigor mortis is well established. There is an aspiration puncture in the right lower chest laterally. A small ulcer, partly healed, is seen on the lateral surface of the left thigh, a little below the iliac crest: the base is of an unusual violet tinge and a brown scab is present.

Internal Examination.

Tongue, pharnyx and oesophagus - Normal.

Thyroid, salivary glands, tonsils - Normal.

Lymph nodes, neck and axillae - Normal.

Pleurae. Left side. Some old adhesions are found near the apex. There is no fluid, but one or two small patches of fibrinous exudate are seen on the diaphragmetic surface.

Right side. There is a little brownish slightly turbid fluid, and on the base and posterior parts is a pleurisy with weak adhesions of yellowish fibrinous exudate.

Trachea and bronchi. A little mucus is seen in the right bronchial tree.

Lungs. Left. Hypostatic congestion, with a few small subpleural abscesses and a septic infarct at the base.
Right. Gross congestion, with many small subpleural abscesses at the base.
Pericardium. A little clear fluid is seen.
Heart. There is agonal thrombus in the right ventricle. Muscle, valves and coronary vessels normal.
Peritoneum. A little brownish fluid is present, and some adhesions around the liver, with fibrinous exudate, are seen.
Stomach and Duodenum. There is an abscess in the wall of the stomach, on the greater curve posteriorly: the mucosa over it shows no gross ulceration.
Small and Large Intestine. Normal. There is no ulceration.
Mesenteric Lymph Nodes. Those near the liver are enlarged and diffluent: the iliac nodes are normal.
Liver. There are about a dozen large abscesses, of 1 to 2 inches diameter, scattered through the organ, which contain greenish pus and are lined by a rough yellowish pseudo membrane. There are a great many smaller abscesses, some of submiliary size, mostly in the right lobe.
Gall Bladder. Normal.
Pancreas. Normal.
Spleen. The organ is enlarged and soft (septic spleen).
Kidneys. There is light congestion and swelling.
Bladder. Normal.
Inguinal Lymph Nodes. Normal.
Brain. There is slight congestion of the meninges, but no abscesses are seen. The cerebrospinal fluid is clear and colourless.

Cause of Death. Suppurative hepatitis.

Histological and Bacteriological Examination.

Pus from several of the liver abscesses consists of necrotic liver tissue with some pus cells: there are many Gram-negative bacilli, which are slender and show a slight tendency to bipolar staining. No amoebae or fungi are seen.

Bacteriological Examination. Culture of the pus from six liver abscesses, from the spleen and from the right pleura all gave pure growths of an organism which appears to be Chromobacterium violaceum. Anaerobic culture and cultures for enteric and dysentery yielded no other organisms.

The organism agrees closely with standard descriptions of Chromobacterium violaceum. It is virulent on intraperitoneal injections into guinea pigs, killing them within 24 hours.

Histological Examination.

Liver. The abscesses contain pus and necrotic liver cells and Gram-negative bacilli are plentiful. No amoebae or acid fast bacilli or fungi are seen. The cellular reaction is predominantly polymorphonuclear, but there are also a moderate number of lymphocytes and a few macrophages. The larger abscesses show early granulation tissue around them. The liver tissue shows gross toxic and fatty change, with occasional small haemorrhages and foci of necrosis.

Kidneys. There is some cloudy swelling.
Spleen. There is swelling with neutrophil infiltration and a few foci of necrosis.
Lung. The basal pleura shows plaques of purulent fibrinous exudate. The adjacent lung is congested and infiltrated mainly with macrophages.
Stomach. There is an abscess, similar to the others, in the submucosa.
Bowel. Ileum and sigmoid colon are normal.
Suprarenal. There is patchy necrosis of the cortex.
Para-aortic Lymph node. There is marked reactive hyperplasia with early abscess formation.
Brain. Cerebral cortex and cerebellum show no abnormality.
Skin ulcer. The ulcer is epithelialised and shows no special features.
Cause of Death. Multiple suppurative hepatitic abscesses caused by C. violaceum.

Protocol 23. Histological examination of Case 2. (No.12 in Sneath et al., 1953, No.15 in Table 14, Chapter XIX, see also Fig.12).

I am indebted to Dr. A.T.H. Marsden, Institute for Medical Research, Kuala Lumpur, Malaya, and to Dr. Joan Ross, Microbiological Research Department, Porton, Wiltshire, for examining sections from this case, and for the following reports.

Dr. Marsden reports:

Brain. No change of importance.
Kidney. No change of importance.
Heart. No change of importance.
Bowel. No change of importance.
Lymph Gland. Hyperplasia of the reticulo-endothelial cells.
Spleen. Congested. There is reticulo-endothelial hyperplasia, with active phagocytosis by macrophage cells.
Skin Ulcer. Shows some chronic inflammation.
Adrenal. Toxic changes with necrosis of some of the cortical cells.
Lung. Generally congested with some tendency to oedema. Patches of fibrinous pleurisy. An abscess is present with much congestion and haemorrhage around it.
Stomach. An abscess is present in the submucosa.
Liver. This is grossly damaged, and there is diffuse round cell infiltration of the portal tracts. There are three types of lesion in the liver, patchy areas of centrilobular necrosis probably due to local stasis, areas of focal necrocis, and the abscesses which characterize this disease. These abscesses consist of a centre of dead parenchyma cells with a few leucocytes surrounded by a rim of polymorphs, lymphocytes, and histiocytes. The characteristic feature is the large amount of necrosis with a comparatively small area of cellular reaction.
Conclusion: This is a blood borne infection of unusual type.

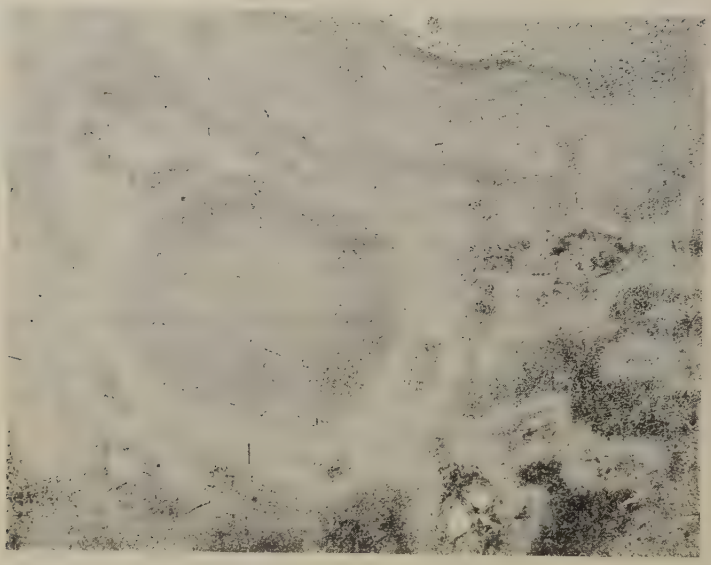


Figure 12a. Photomicrograph of section of liver abscesses of Case 2. x 20.

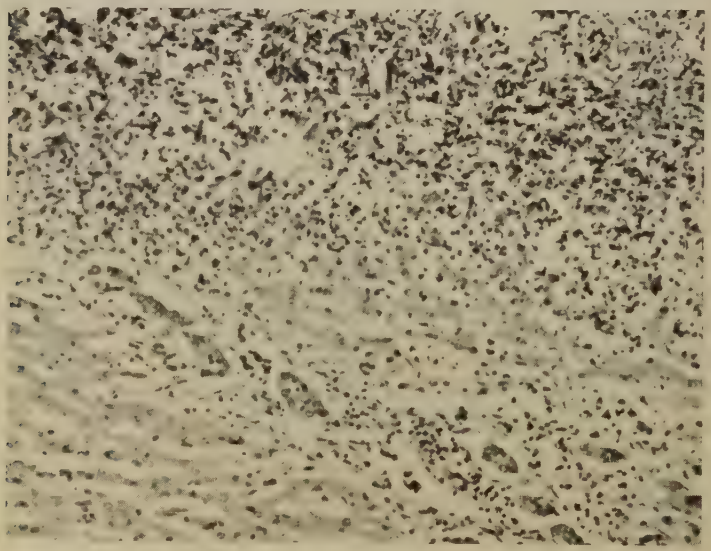


Figure 12b. Photomicrograph of edge of liver abscess of Case 2. x 65.

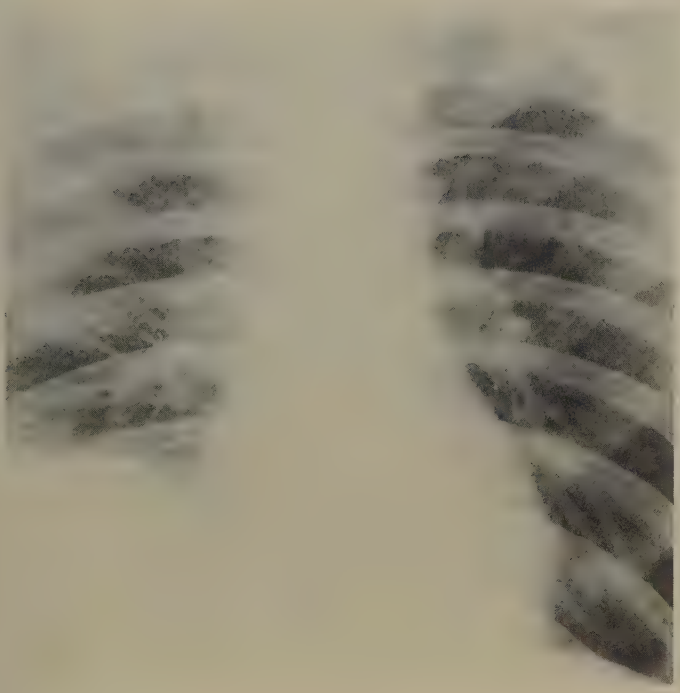
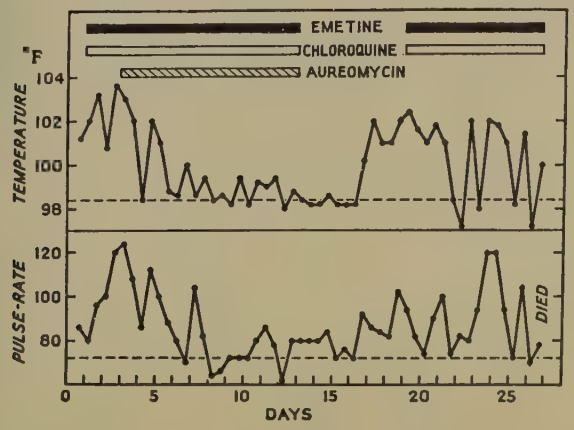


Figure 13. Chest X-ray of Case 2 taken the day before death, showing raised right diaphragm.



Treatment and course of fatal infection by *Chr. violaceum*.

Figure 14. Clinical chart of Case 2 (from Sneath et al., 1953).

Dr. Ross reports:

Lung: This shows gross inflammatory oedema with fibrinopurulent pleurisy. Gram-negative bacilli are numerous in the cellular areas in the pleural exudate and stain well with eosin methylene blue.

Liver: Some periportal fibrosis with varying amounts of inflammatory infiltration. There are scattered areas of acute necrosis of liver tissue with replacement by granulation tissue and polynuclear and mononuclear cells. Gram-negative short stout bacilli are present. One abscess is well encapsulated with a greater proportion of plasma cells and macrophages, some vacuolated.

Protocol 24. Bacteriological examination of the strain of Chromobacterium isolated from Case 2.

This was a preliminary examination of the organism by routine methods.

I. Isolation

Pus from liver abscesses showed on microscopic examination no amoebae, fungi or acid fast bacilli. There were about equal numbers of pus cells and of necrotic liver cells, among which were many Gram-negative bacilli, measuring about 0.7 μ by 2.0 μ; they showed slight bipolar staining, and were generally single, occasionally as diplobacilli. No capsules were visible. Cultures of the pus were made on blood agar plates both aerobically and anaerobically at 37°. They both yielded pure growths of Chromobacterium violaceum. Cultures from selenite broth plated onto MacConkey agar yielded no Salmonella or Shigella organisms and only a few colonies of Chromobacterium. Saboraud's glucose agar gave no growth of fungi after four weeks at room temperature (circa 30° C), and Lowenstein-Jenner medium yielded no growth of acid fast bacilli after four weeks at 37°. The spleen gave a pure growth of Chromobacterium, as did the exudate on the lungs. A few bacilli were visible in smears from the spleen. Scrapings from the partly healed skin ulcer on the left thigh yielded no colonies of Chromobacterium, but only white staphylococci and diphtheroid bacilli.

A guinea pig was inoculated intraperitoneally with 1 ml of pus from a liver abscess: it sickened on the 4th day and died on the 7th day. Pure cultures of Chromobacterium were isolated from the pleural and peritoneal cavities. The animal showed an abscess in the abdominal wall at the site of inoculation, acute fibrinous peritonitis, acute sero-sanguinous pleurisy and enlargement of the spleen, but no liver or lung abscesses. 0.5 ml of the peritoneal fluid was injected into a second guinea pig which died of septicaemia in 24 hours, and Chromobacterium was recovered on blood agar in pure culture from its blood.

A mouse was also inoculated intraperitoneally with 0.1 ml of pus from a liver abscess of the patient: it died after 10 days, and showed emaciation, slight jaundice, fibrinous peritonitis and small abscesses of the liver and spleen from which Chromobacterium was isolated in pure culture on blood agar.

II. Bacteriological examination of the organism (strain BN).

The bacterium was a Gram-negative motile rod, about 0.7 x 2 μ, without capsules or spores, showing slight bipolar staining. Nutrient agar cultures showed, after 24 hours at 37° aerobically, round convex shiny colonies about 1 mm in diameter of a pale violet colour. After 2 days they were larger and deep violet. Anaerobic cultures were similar but colonies were smaller and colourless. On blood agar there was hazy haemolysis after 2 days. In broth it gave a violet ring at the surface, with moderate turbidity and a fragile pellicle. It grew on MacConkey agar giving violet colonies. It grew better at 37° than 30°. The pigment was insoluble in water, chloroform or benzene but soluble in ethanol.

In peptone water sugars it produced no gas, but acidity in glucose, not in maltose, lactose, salicin, mannitol, or sucrose. It was indole-negative, MR-doubtful, VP-negative, and catalase positive. Tests with paper discs suggested that it was sensitive to the antibiotics streptomycin, chlortetracyclin, oxytetracyclin, and chloramphenicol, but resistant to penicillin.

III. Animal inoculation experiments.

Guinea pigs. Three guinea pigs were used. They were inoculated intraperitoneally with 0.1 ml, 0.01 ml, and 0.001 ml, respectively, of a 24 hour nutrient broth culture of the organism. The two animals which received the larger inocula died within 24 hours, while the animal which received 0.001 ml of culture died after 42 hours. A control experiment was made by injecting a guinea pig with 0.1 ml of the culture which had been killed by heating at 100°C for 5 minutes; it showed no ill effects and was alive two weeks later. The animals which died all showed evidence of acute septicaemia, since the organism was recovered in pure culture from heart blood. The only post-mortem signs were slight turbid peritoneal effusion with peritoneal congestion, a little clear pleural effusion and an area of gelatinous oedema in the subcutaneous tissue at the site of inoculation.

Another guinea pig was inoculated intraperitoneally with 0.5 ml of the peritoneal effusion from one of the dead animals, and it died of septicaemia within 24 hours. The organism was recovered in pure culture from the heart blood.

Mice. Two mice injected intraperitoneally with 0.1 ml of a 24-hour broth culture both died within 12 hours, and the organism was recovered in pure culture from the heart blood.

IV. Serological experiments.

The strain of Chromobacterium violaceum was not agglutinated on slide testing by the routine antisera to the commoner pathogenic bacteria, comprising the following antisera: Polyvalent Salmonella H specific and nonspecific antiserum, O antisera to typhoid, paratyphoid A, paratyphoid B, and paratyphoid C, antisera to Shiga, Sonne, Flexner (polyvalent) and Boyd (polyvalent) dysentery bacilli, antisera to Brucella abortus, Vibrio comma, and Whitmore's bacillus. Unfortunately no sample of blood from the patient was preserved for agglutination tests with the Chromobacterium strain.

Protocol 25. Further virulence tests on the strain from Case 2.

Dr. J. P. F. Whelan and Dr. R. Bhagwan Singh of the Institute for Medical Research, Kuala Lumpur, Malaya, carried out some more investigations with the strain BN, and have kindly given me the following summary.

I. Guinea pigs.

A guinea pig was inoculated subcutaneously with 0.5 ml of pus from a liver abscess of the patient. It developed an abscess at the site of inoculation, but otherwise it appeared healthy. It was killed 23 days later; the abscess contained cheesy pus from which Chromobacterium violaceum was recovered in pure culture. The regional lymph nodes were enlarged but contained no pus. A sample of serum was found to agglutinate a heat killed suspension of the Chromobacterium strain to a titre of 680.

A guinea pig inoculated with pus intranasally remained well.

Two guinea pigs were inoculated intraperitoneally with 1 ml of a broth culture: both died of acute septicaemia within 24 hours. The organism was isolated from the blood and the urine of both. Four guinea pigs were inoculated subcutaneously with a broth culture. Two animals received 1 ml and two 0.7 ml. The two which received the larger inoculum died of acute septicaemia within 24 hours. One of the animals which received 0.7 ml became very sick and developed an ulcer at the site of inoculation, which healed after three weeks, and the animal recovered: while it was very sick a sample of heart blood yielded a growth of Chromobacterium violaceum. The other animal which received 0.7 ml died after four days; post-mortem examination showed acute inflammation and gelatinous oedema over a wide area around the inoculation site, and multiple small abscesses in the liver, spleen and lungs. There was some turbid peritoneal and pleural exudate. Chromobacterium violaceum was isolated from the abscesses.

A guinea pig was scarified on the abdomen and some drops of a broth culture was rubbed on. The animal remained healthy.

Two guinea pigs were given large quantities of a broth culture mixed with their food for three days. They remained healthy.

To test for the presence of an exotoxin, a 48-hour broth culture was Seitz-filtered, and two guinea pigs were inoculated with 1 ml of the filtrate subcutaneously. They showed no ill effects. Therefore, no potent endotoxin was present.

II. Rabbits.

Two rabbits were injected intravenously with 0.5 ml of a broth culture. They both died within 24 hours of acute septicaemia.

Protocol 26. Clinical summary of Case 3.
(No.13 in Sneath et al., 1953, No.16 in Table 14, Chapter XIX)

This summary was kindly provided by Major D. Edwards, R.A.M.C.

The patient was a British soldier aged 20 years. He was admitted to British Military Hospital, Kamunting, Taiping, Malaya, on 20th January 1952, complaining of headache and malaise. On examination temperature 102°F and slight liver tenderness, no other abnormal findings. Treated with penicillin (600,000 units ó hourly i.m.i.) for 7 days, but remained unwell. White blood count 15,600 per cu mm; Neutrophils 75%, lymphocutes 22%, Monocytes 2%, Eosinophils 1%. No serum agglutinins for Brucella or scrub typhus. Urine: normal.

The pyrexia subsided and penicillin was discontinued but on 7th February he was again febrile, and the liver was tender (but not enlarged). A tentative diagnosis of amoebic hepatitis made, though no amoebae were found in the faeces. Treated with emetine 65 mg daily for 12 days. On 14th February mild diarrhoea developed: the stool contained a few red corpuscles and leucocytes and on culture yielded an almost pure growth of Chromobacterium violaceum. The pyrexia and liver tenderness slowly subsided. On 11th Marsh he was sent on convalescence and on 26th March he appeared fit and was discharged. The stools then did not yield Chromobacterium on culture.

I was sent this strain of Chromobacterium. It was a typical mesophilic strain, but was unfortunately lost. The faeces were plated on MacConkey agar and besides the violet colonies, there were a few coliforms and enterococci.

Protocol 27. Clinical summary of Case 4.
(No.14 in Sneath et al., 1953, No.17 in Table 14, Chapter XIX)

This summary was kindly provided by Major H.G.D. Hetherington, R.A.M.C.

The patient was a British soldier aged 35 years. He was admitted to British Medical Hospital, Kamunting, Taiping, Malaya, on 19th February 1952. He had had diarrhoea for 9 days, with much mucus in the stools and occasionally a little blood. There was abdominal tenderness but no other abnormal finding. Cultures of stool showed no pathogenic bacteria. The white blood count was 7,800 per cu mm with 69% of polymorphs, 26% lymphocytes, 3% of monocytes and 2% of eosinophils. On 21st February some amoebae resembling Entamoeba histolytica were seen in the stools, but there were no red blood cells. He was treated with emetine 65 mg daily, followed by a course of emetine bismuth iodide and discharged early in April. On 4th June he was readmitted complaining of acute diarrhoea, but recovered the next day and was discharged on 8th June. Stools showed no amoebae or [bacterial] pathogens. On 9th August 1952, he was readmitted again complaining of intermittent acute diarrhoea, with visible blood in the stools. There were no abnormal physical signs.

Sigmoidoscopy showed an increase in mucus and occasional pitted scars suggesting healed amoebiasis. There was no fever or leucocytosis (white blood count 7,000 per cu mm; polymorphs 45%, lymphocytes 39%, monocytes 4%, eosinophils 2%). A stool specimen showed large numbers of red blood cells, and on culture the predominant growth was Chromobacterium violaceum. He was treated with aureomycin (chlortetracycline) 0.25 gram four times a day for three days. The diarrhoea ceased and on 20th August culture of the stools showed no pathogens and no colonies of Chromobacterium. He was readmitted on 29th September, complaining of a recurrence of diarrhoea with a little blood in the stools. On sigmoidoscopy some amoebic ulcers were seen, and he was given a further course consisting of emetine 65 mg daily and carbazone 260 mg twice a day for 10 days followed by diodoquine 600 mg three times a day for 21 days. After this treatment his bowel appeared normal on sigmoidoscopy and he was discharged.

This culture of Chromobacterium was not kept: it was a typical mesophilic strain. The faeces were plated on MacConkey agar and the violet colonies predominated. It was purified and briefly studied: the pigment had the solubility characteristics of violacein.

APPENDIX II

A REVIEW OF THE BLUE AND VIOLET CHROMOGENIC BACTERIA

I Bacteria only chromogenic on unusual media (Nos. 1-9).

II Bacteria which are not blue or violet chromogens but which have been confused with them on account of their names (Nos. 10-16).

III Bacteria which are not strains of Chromobacterium or which are of doubtful affinities. (Nos. 17-77)
- a. Chromobacterium viscosum Grimes (No. 25) and bacteria possibly related to it. (Nos. 17-26)
- b. Species of Pseudomonas. (Nos. 27-33)
- c. The "indigo bacteria." (Nos. 34-39)
- d. Species of Micrococcus. (Nos. 40-42)
- e. Probably species of Corynebacterium. (no. 43)
- f. Purple variety of Pasteurella pestis. (No. 44)
- g. Bacteria of uncertain genus. (Nos. 45-77)

IV Bacteria which appear to be strains of Chromobacterium. (Nos. 78-142)
- a. Bacteria recognizable as mesophilic strains of Chromobacterium. (Nos. 78-91)
- b. Bacteria recognizable as psychrophilic strains of Chromobacterium. (Nos. 92-113)
- c. Chromobacterium, but of uncertain species. (Nos. 114-142)

V Check list of epithets used in combination with the generic name Chromobacterium.

In this appendix are described all the blue and violet chromogenic bacteria which I have been able to find in the literature, with the exception of purely derivative work (such as descriptions in text-books which are obviously derived from other published sources) and a few groups which are clearly distinct from chromobacteria and are unlikely to be confused with them (e.g. actinomycetes and the purple sulfur bacteria and photosynthetic bacteria).

The synonymy has been given as fully as possible for strains of Chromobacterium and indigo bacteria, and in less detail for other organisms; however, the names used in the latest complete edition of Bergey's Manual of Determinative Bacteriology (6th ed., Breed et al., 1948) have been included where possible for ease of reference. The bacteria are described under their original names, whether legitimate or not, unless there is sound evidence for preferring another name. Within the sections and subsections they are arranged alphabetically by specific epithets (not by generic names). It is emphasized that the sections and subsections are only provisional, and they are not intended as taxa, with the exception of Section IV which is the genus Chromobacterium sensu stricto. Each organism has been given a number for ease of reference: some bacteria found in the literature since the composition of the bulk of the work have been interpolated to avoid renumbering.

I. BACTERIA ONLY CHROMOGENIC ON UNUSUAL MEDIA

No. 1. Pseudomonas Beijerinckii Hof, 1935, p. 152. (Pseudomonas beijerinckii Breed et al., 1948, p. 109; Haynes in Breed et al., 1957, p. 121). Modern Latin genitive of noun, named for M. W. Beijerinck.

This bacterium is a halophilic pseudomonad isolated from salted beans, which it may discolour violet. Kluyver, Hof and Boezaardt (1939) found that the purple diffusing pigment is formed from meso-inositol (which is present in beans), and that it is probably the calcium salt of tetrahydroxyquinone. The pigment is insoluble in alcohol and is only formed under microaerophilic conditions since it is oxidized to a colourless compound by free oxygen. Hof was able to isolate the organism regularly from salted beans, whether they were discoloured or not, by plating on bean extract agar or on agar containing meso-inositol. The name is validly published.

No. 2. Bacillus belfantii Carbone and Venturelli, 1925, p. 60. (Clostridium belfantii Spray in Bergey et al., 1939, p. 759; Breed et al., 1948, p. 803; Spray et al. in Breed et al., 1957, p. 679). Named for Belfant, an Italian bacteriologist.

This, like Nos. 3 and 6, was an anaerobic motile spore-forming bacillus. The spores were oval and central. The colonies on agar were colourless, but in potato mash medium it produced a violet coloured foam. The name is validly published.

No. 3. Bacillus de rossii Carbone and Venturelli, 1925, p. 65. (Clostridium derossii Spray in Bergey et al., 1939, p. 760; Breed et al., 1948, p. 803). Named for G. de Rossi, an Italian bacteriologist.

This bacillus was very similar to No. 2 above. The name is validly published.

No. 4. Mycobacterium globerulum Gray, 1928, p. 265. (Nocardia globerula Waksman and Henrici in Breed et al., 1948, p. 903). Latin adjective, globular.

This organism from soil was a Gram-positive rod, sometimes forming filaments which break into coccoid forms. Gelatin was not liquefied. It utilizes phenol for growth and oxidizes indole to indigotin, giving blue colonies on indole agar. The name is validly published.

No. 5. Pseudomonas indoloxidans Gray, 1928, p. 263. (Pseudomonas indoloxidans Breed et al., 1948, p. 698; Haynes in Breed et al., 1957, p. 117). Modern Latin participle, indole-oxidizing.

This was a small Gram-negative rod with lophotrichous flagella which

produced blue crystals of indigotin on indole agar. From soil. The name is validly published.

No. 6. Bacillus maggiorai Carbone and Venturelli, 1925, p. 62. (Clostridium maggiorai Spray in Bergey et al., 1939, p. 759; Breed et al., 1948, p. 803). Presumably named for Maggiora, an Italian bacteriologist.

This was very similar to No. 2 above. The name is validly published.

No. 7. Bacillus mesentericus.

Lasseur and Thiry (1913) reported that some strains produced reddish or violet pigments upon asparagin-glycerol media. Godfrin (1934, p. 141 et seq.) gives more details. The organism was a Gram-positive spore-forming rod, and the varieties are referred to as B. mesentericus fuscus Flügge and B. mesentericus niger Lunt. It is doubtful whether they can be clearly recognized now, but they may have been strains of the Bacillus subtilis group (see Smith, Gordon and Clark, 1952, p. 72 et seq.). Recently, Garibaldi and Neilands (1956) have reported coloured iron compounds which are produced under certain conditions by Bacillus subtilis and Bacillus megaterium: they are probably polyhydric phenols, and the iron compound from Bacillus subtilis is purple in colour and has been identified as the ferric complex of 2, 3 dihydroxybenzoylglycine (Ito and Neilands, 1958). Probably Lasseur and Thiry were studying these compounds.

No. 7a. Arthrobacter oxydans Sguros, 1954, p. 21. (Arthrobacter oxydans Breed et al., 1957, p. 608). Modern Latin, oxidizing.

This organism produces a blue diffusing pigment from nicotine. It is a Gram-negative nonmotile pleomorphic rod. The name is validly published.

No. 8. Micrococcus piltonensis Gray and Thornton, 1928, p. 81. (Micrococcus piltonensis Gray, 1928, p. 266; Breed et al., 1948, p. 271). Etym. dub.; presumably from Pilton, village in England.

Gray (1928) refers to this as one of the three organisms which produced indogotin from indole (see No. 4 and No. 5). It seems to have been a Gram-positive micrococcus. The name was validly published by Gray and Thornton.

No. 8a. Vibrio purpureus Kadota, 1951. p. 59, and Pls. 2, 3, 4. Latin adjective, purple-coloured.

This was described as a new species of marine agar-digesting bacterium. It produced a dark purple growth on a medium composed of agar and inorganic salts, but no pigment on ordinary media. The pigment is not further described. The organism also digested cellulose. It was a

curved rod with a single polar flagellum, Gram-negative, nonsporing, and with weak fermentative powers on carbohydrates. It was a facultative anaerobe and reduced nitrate to nitrite and to gaseous nitrogen. The name is validly published. Professor C.B. van Niel (personal communication) suggests it may be very similar to Microspira tyrosinatica of Beijerinck (see Breed et al., 1948, p. 202).

No. 9. Clostridium sp. McClung, 1942, p. 117.

McClung isolated this on corn-grains incubated anaerobically. It was a strict anaerobe producing spores and a blue-green pigment which faded on exposure to air. No specific epithet was given to it.

II. BACTERIA WHICH ARE NOT BLUE OR VIOLET CHROMOGENS BUT WHICH HAVE BEEN CONFUSED WITH THEM ON ACCOUNT OF THEIR NAMES.

No. 10. Bacillus azureus Zimmerman, 1893, p. 100.
(Bacillus azureus Migula, 1900, p. 785; Breed et al., 1948, p. 649, not Bacillus azureus Matzuschita, 1902, p. 308).
Latin adj., sky-blue.

Zimmermann named this bacillus on account of the blue iridescence of its colonies. It formed no pigment. Lehmann and Neumann (1899, Vol. 2, p. 248) thought that it was a coliform. Matzuschita (1902, p. 308) describes a different organism under this name (No. 45a), giving sky-blue colonies containing blue pigment, giving brown growth on potato and not liquefying gelatin. The name is validly published.

No. 11. Bacterium cyaneus [sic] White, 1905, p. 16 and Tab. p. 28).
(Micrococcus cyaneus White, 1906, p. 16; Breed et al., 1948, p. 695. Not Bacterium cyaneum Leonard, 1904, p. 398 (No. 21), not Bacteridium cyaneum Schroeter, 1872, pp. 122, 126 (No. 56). Latin adj., blue.

A nonmotile nonsporing Gram-positive coccobacillus producing on agar a pink diffusing pigment. The colonies were yellow. It liquefied gelatin, produced no acid from glucose, grew at 37° and was indole negative. On potato it was yellowish. From bees. The name is a later homonym of Leonard's bacillus.

No. 12. Bacillus glaucus Eisenberg, 1891, p. 78.
(Bacillus glaucus Roux, 1892, p. 300; Lustig, 1893, p. 106; Sternberg, 1893, p. 637; Kruse in Flügge, 1896, Vol. 2, p. 313; Matzuschita, 1902, p. 323; Breed et al., 1948, p. 657. Bacterium glaucus [sic] Chester, 1897, p. 116. ? Grauer Bacillus Maschek, 1887, p. 74; Adametz, 1888, p. 56; ? Bacillus canus Migula, 1900, p. 711). Latin adj., sea-green.

This organism was probably based on the Grauer Bacillus of Maschek, although Eisenberg does not make this clear. Maschek and Eisenberg

described its colonies as grey, with a brown edge. It slowly liquefied gelatin and gave a grey growth on potato. It was isolated from water. Lustig described the colonies as deep blue, but this is evidently an error. The name is validly published.

No. 13. Klebsiella indigogena Trevisan, 1888, cited in Trevisan, 1889, p. 25.
(Klebsiella indigogena Trevisan, 1889, p. 25; de Toni and Trevisan in Saccardo 1889, p. 1033. Bacillus indigogenus Eisenberg, 1891, p. 364; Breed et al., 1948, p. 659. Bacillus Indicans [sic] Oliver, 1902, p. 651; Bacille indigogene, Alvarez, 1887, p. 287). Latin, indigo-producing.

This was not a chromogen; it produced indigo from indican when fermenting extracts of the in igo plant and was probably a Klebsiella.

No. 14. Bacillus nubilus Frankland and Frankland, 1889, p. 386, Taf. III, figs. 1A-1D.
(Chromobacterium nubile Ford, 1927, p. 472. Corynebacterium nubilum Jensen, 1934, p. 44; Breed et al., 1948, p. 404).
Latin adj., cloudy, not nubilis, marriageable.

Agar slope cultures showed a violet sheen but no pigment. The name is validly published.

No. 15. Spirillum violaceum Warming, 1875, p. 325, Tab. VII, fig. 3.
(Spirillum violaceum Winter in Rabenhorst, 1881, Vol. 1, p. 65; de Toni and Trevisan, 1889, in Saccardo, 1889, p. 1013. Thiospirillum violaceum Migula, 1900, p. 1050; Breed et al., 1948, p. 852; van Niel in Breed et al., 1957, p. 47). Latin adj., violet-coloured.

This was evidently a purple sulfur bacterium, as the cells contained sulfur granules and were violet. The name is validly published.

No. 16. Thermobacillus violaceus Feirer, 1927, p. 52.
(Thermobacillus violaceus Breed et al., 1948, p. 735.)
Latin, violet-coloured.

This was a thermophilic spore-forming bacillus which turned litmus milk violet. Clarke and Tanner (1938) mention a similar bacillus under the name of Bacillus violaceus, but did not commit themselves as to its true name. It was possibly the same strain. The name is validly published.

III BLUE OR VIOLET CHROMOGENIC BACTERIA WHICH ARE NOT STRAINS OF CHROMOBACTERIUM OR WHICH ARE OF DOUBTFUL AFFINITIES.

III (a) Chromobacterium viscosum Grimes (No. 25) and bacteria possibly related to it.

The bacteria of this group (which may form a natural taxon but which need further study) are nonsporing rods which produce a diffusing blue pigment, generally best on media containing carbohydrates. They are generally Gram-positive, nonmotile, and strictly aerobic.

No. 17. Bacillus budapestinensis α Ajtai, 1897, p. 659 and VI táb 5, 6, sz. rajzával. From Latin, of Budapest.

This bacterium was a weakly Gram-positive rod which produced a blue diffusing pigment, scantily on nutrient agar and abundantly on glycerol agar. It was aerobic and nonsporing.

No. 18. Bacillus budapestinensis β Ajtai, 1897, p. 662. From Latin, of Budapest.

This organism was very similar to Bacillus budapestinensis α, and was probably a variety of it. The descriptions are not very full and there is some doubt whether they are closely related to Chromobacterium viscosum Grimes. The name is probably validly published, since α and β seem to refer to two varieties.

No. 19. Bacterium coelicolor Miller, 1908, p. 195. (Bacillus coelicolor Godfrin, 1934, pp. 33, 213. Bacterium coelicolor Breed et al., 1948, p. 401. Mycobacterium coelicolor Krassilnikov, 1941, p. 100). From Latin, sky-coloured.

This organism was described by Müller as a diphtheroid. It produced a diffusing blue pigment on potato and media containing milk or starch, but little or none on nutrient agar, or agar containing glucose and other simple carbohydrates. The water-soluble pigment was called amylocyanine, and Müller believed that it was the same as a pigment produced by another organism he described, Streptothrix coelicolor. The pigment became red with acids and with magnesium sulfate and green with alkalis. It was insoluble in fat solvents.

The bacterium was a nonmotile nonsporing rod showing barred staining and occasional metachromatic granules. It was Gram-positive and strictly aerobic. The optimum temperature was 22°-25°; at 36° it grew well but produced no pigment. The growth upon glucose agar was viscid. On potato it formed a blue growth and the potato was stained bright blue. It liquefied gelatin and coagulated serum slowly, was nonhaemolytic, and produced acid from glucose, sucrose and glycerin but not from the other commonly used carbohydrates. Müller noted its similarity to Bacteridium cyaneum Schroeter (No. 56) but commented that the pigment of the latter became blue with alkalis, not green, although Schroeter described

a variety (Micrococcus pseudo cyaneus Cohn, No. 65) whose pigment became green with alkali.

Müller's organism seems to have been very similar to Chromobacterium viscosum Grimes (No. 25), the main points of difference being that the former was reported as indole-positive and that the conditions for pigmentation are somewhat different. The name is validly published.

The pigment of Streptothrix coelicolor has been studied by Sánchez-Marroquín and Zapata (1954).

No. 20. Bacillus Coerulefaciens McFarlane, 1895, p. 935. (Bacillus Coerulefaciens McFarlane in Norris and Oliver, 1897, Vol. 2, p. 492). Latin, sky-blue making.

This organism was isolated from the conjunctiva but was not associated with any disease. It was a nonsporing, slender Gram-positive rod which was motile. It slowly liquefied gelatin and turned milk alkaline. The colonies on agar were yellowish but on potato it produced at room temperature (but not at 37°) a diffusing blue pigment. Except for motility it was similar to Chromobacterium viscosum (No. 25). The name is validly published.

No. 21. Bacterium cyaneum Leonard, 1904, p. 398. (not Bacterium cyaneus [sic] (No. 11) White, 1906, p. 16, not Bacteridium cyaneum (No. 56) Schroeter, 1872, pp. 122, 126). Latin adj., blue.

This organism was a Gram-positive, nonmotile, nonsporing rod producing a diffusing blue pigment, especially on glucose-containing media. The pigment was insoluble in chloroform. It was an obligate aerobe, liquefied gelatin and digested coagulated serum. It produced no gas from carbohydrate media (acid production is not recorded) and grew best at room temperature. There was no growth at 10°, and at 37° it grew slowly but produced no pigment. On potato it produced a greenish growth, while the medium was stained blue. Like Chromobacterium viscosum Grimes it showed polar granules and although it had no definite capsule it produced a viscid growth. It seems to be very similar to that organism, the main point of difference being that the appearance of the pigment in plate cultures was dichroic (blue by transmitted light and red by reflected light). I have not been able to convince myself that Chromobacterium viscosum (No. 25) shows this effect. The name is validly published.

No. 22. Bacillus polychromogenes Thiry, 1900, p. 9. (Bacillus polychromogenes Chamot and Thiry, 1900, p. 378; Macé, 1901, p. 912; 1913, vol. 2, p. 406; Godfrin, 1934, pp. 80, 228; Breed et al., 1948, p. 233. Bacterium polychromogenes Lehmann and Neumann, 1920, Vol. 2, p. 406. Bacille polychrome, Thiry, 1896, p. 885; Macé, 1897, p. 849; 1898, p. 58 and Pl. 29. Bac. polichromo [sic] Calderini, 1925, p. 772). From Greek, forming many colours.

This organism was isolated from water. It was an encapsulated pleomorphic Gram-positive rod, showing occasional branching and metachromatic granules. Macé (1901, p.912) said it was often club-shaped and its motility was doubtful. It liquefied gelatin, peptonized milk, was indole-negative and gave on potato a deep blue diffusing pigment. On gelatin cultures were at first green, then red, then blue-violet. The blue pigment was soluble in water, not in chloroform or in alcohol. It became reddish-violet with acids and blue-green with alkalis, and in neutral aqueous solution it had an absorption peak at 594 mμ.

Despite Thiry's full description, the systematic position of this organism is puzzling. The behaviour in culture is reminiscent of that shown by Chromobacterium viscosum and Bacillus visco-fucatus (No. 25). It may therefore belong to this group, Thiry's name is validly published.

No. 22a. Micrococcus polychromus Makarova, 1949, cited by Krassilnikov, 1949, p.270. From Greek, many coloured.

I have not been able to consult the original description. From the very brief description of Krassilnikov it may be of this group, but was a large coccus, producing a blue-green pigment which diffused on media containing carbohydrates. The pigment is decolorized by alkali. It liquefied gelatin and did not reduce nitrates.

No. 23. Bacillus violaceus sacchari Agar, 1894, p. 265. (Bacterium violaceous [sic] sacchari Chester, 1901, p.260; Breed et al., 1948, p.647. ? Bacillus violaceus sacchari Dyar, 1895, p.369). Latin, violet coloured, M. Latin, of sugar; Modern Latin, violet bacillus of sugar.

The organism described by Agar was a nonmotile, nonsporing short bacillus which liquefied gelatin. It coagulated milk and on ordinary media produced no pigment. It produced acid from lactose and did not reduce nitrates. On media containing carbohydrate it produced a purple diffusing pigment. Old milk cultures became violet. It was isolated from air. It was a strict aerobe and not pathogenic on inoculation into rabbits. The pigment was insoluble in alcohol.

Dyar isolated an organism from air which he considered to be identical, but it differed in several respects and is described separately later (No. 69).

Agar's bacillus was very probably of this group, though it is by no means certain. Agar's name is an invalid trinomial.

No. 24. Bacterium visco-fucatum Harrison and Barlow, 1905, p.97; 1906, p.517.
(Bacillus visco-fucatus Harrison and Barlow, 1905, p.97; 1906, p.517. Bacillus viscofucatus Godfrin, 1934, pp.75, 227. Chromobacterium viscofucatum Bergey et al., 1923, p.121; 1925, p.127; 1930, p.162. Chromobacterium viscofucatum Bergey et al., 1934, p.174; 1939, p.94; Breed et al., 1948, p.234). Latin adj., viscid; part., painted.

This was a Gram-positive pleomorphic encapsulated bacillus. It was nonmotile, nonsporing, and showed barred staining and metachromatic granules. Some branched forms were seen. It was strictly aerobic and produced abundant viscid slime. On most media containing carbohydrates it produced a diffusing blue pigment which was soluble in water but not in chloroform. The pigment became red with acids, blue-green with alkalis and violet with magnesium sulfate solution. Pigment granules were reported to be present, but in unpigmented cultures on media without carbohydrates there were deposits of crystals and it is possible that the pigment granules were those crystals which had absorbed pigment. The pigment was evidently slightly dichroic as it appeared violet in some circumstances. The organism grew at 0° and at 39° slowly, with an optimum at about 30°. It grew upon mddia containing 8% of sodium chloride. On potato it gave a pale growth, though the medium was stained deep blue. On some media it gave a greenish pigment (e.g. galactose gelatin) and in old cultures reddish tints predominated. Its characters are in close agreement with those of Chromobacterium viscosum (No. 25). The name would appear to be validly published although Harrison and Barlow were uncertain of the correct generic name.

No. 25. Chromobacterium viscosum Grimes, 1927, p. 368. (Chromobacterium viscosum Bergey et al., 1930, p. 161; 1934. p. 173; 1939, p. 94; Breed et al., 1948, p. 234; Gilman, 1953, p. 48; Sneath, 1956b, p. 71). Latin adj., viscous.

This is the strain here called GR and is NCTC 2416. It is an encapsulated nonmotile, nonsporing Gram-positive bacillus which was isolated from butter. It produces a diffusing violet-blue pigment but only upon media containing carbohydrates. Gilman (1953) considered it to be close to Chromobacterium iodinum Davis (No. 61), and suggested that the pigment might be a phenazine and that it might be transferred to Pseudomonas. He found it to be catalase positive, to reduce nitrates and to utilize citrate for growth. Sneath (1956b) considered it to be close to the diphtheroid bacilli, and found that it did not utilize citrate for growth; he named the pigment viscosin (not the viscosin of Kochi, Weiss, Pugh and Groupé, 1951) and observed that in acid solution it showed absorption peaks at about 330 and 566 mμ and transmittance peaks at about 320 and 510 mμ, in reasonable agreement with Gilman's observations. The organism does not produce chitinase (Clarke and Tracey, 1956). The name is validly published.

I have studied this strain in some detail and a description is appended. It is difficult to know to which genus it belongs. It is clearly not a member of the genus Chromobacterium, nor closely related to C. iodinum Davis. A recent study (Sneath and Cowan, 1958) suggests that it is, apart from its morphology, closer to some micrococci than to the diphtheroids.

Description of Chromobacterium viscosum Grimes strain GR (NCTC 2416 and ATCC 6918). Unless otherwise stated the methods are those used for Chromobacterium, and detailed results are given in the Protocols in Appendix I.

Morphology. A strongly Gram-positive rod, about 0.8 x 1.5 μ, pleomorphic with many clubbed forms, with marked barred staining, metachromatic granules, not acid fast, nonmotile, nonsporing, with a definite capsule, with much fat in young cultures, arranged in irregular or palisade-like clumps. Old cultures are Gram-variable.

Cultural behaviour. Colonies on nutrient agar are round, convex, smooth, shiny, with entire edge, semiopaque greyish and viscid, and no pigment is formed. No haemolysis on blood agar. Nutrient agar slant at 25° (2 days) shows a grey semiopaque viscid smooth growth with lobate edge and no pigment. Gelatin stab (7 days at 20°) shows moderate stratiform liquefaction with scanty filiform growth in the depth and no pigment. Nutrient broth cultures show slight turbidity, no ring or pellicle, slight deposit. Löffler's serum slope shows a grey shiny growth with faint tinge of blue diffusing pigment, and no digestion in 14 days. Potato (25°) shows a raised creamy-yellow growth, and after 10 days a pale sky-blue pigment diffuses through the potato, the growth itself remaining yellow. Pigment is formed on some other media (see below).

Resistance. It is mildly thermoduric: some viable organisms are found in a loopful of broth culture after 15 mins. at 56°, but cultures are sterile after 30 mins. It has the usual resistance to phenol. It is penicillin sensitive.

Metabolism and nutrition. It is fairly strictly aerobic, grows from 10° to 37°, (optimum about 35°), grows from pH 6 to pH 9, and grows on 6.5% NaCl agar. It produces no HCN. No growth in Koser's citrate or Simmons' citrate agar with yeast.

Carbohydrate tests. It oxidizes glucose, fructose, mannose, sorbitol, sucrose, trehalose, lactose, galactose, cellobiose, maltose, not L(+)-arabinose, D(+)xylose, salicin, mannitol, inulin, glycerol, starch, or m-inositol (using Hugh and Leifson media). After about 10 days some acid is formed anaerobically from glucose. In peptone water with carbohydrates no definite acidity is detectable.

Biochemical. Litmus milk: slight acid, a small clot, bleaching of litmus, some peptonization. Indole -, NH_3 -, M.R. -, V.P. -, H_2S -, M.B. reduction -, nitrate reduction +, nitrite destruction weak, catalase +, urease -, phosphatase strong positive, aesculin hydrolysis weak (moderate in plates, poor in liquid medium), casein hydrolysis weak, gelatin hydrolysis strong positive, starch hydrolysis -, haemolysis -, egg-yolk reaction -, malonate utilization -, phenylpyruvate -, arylsulfatase -, chitin digestion -.

Pigment. On 1% peptone agar containing 0.5% of NaCl and of glucose, sucrose, or mannitol there is after 1 day at 25° a little green diffusing pigment. After several days there is a large amount of a diffusing blue-violet pigment. The blue-violet pigment from sucrose peptone agar was extracted with water. It became red in 0.1 M HCl and blue-green in 0.1 M NaOH. Addition of saturated $MgSO_4$ made it purple. In phosphate buffer of pH 7.0 there was an absorption maximum at 574 mμ and minimum at 472 mμ (see Fig. 11). In phosphate buffer of pH 4.2 the maximum was at 566 and the minimum at 468 mμ. At both pH there were small peaks at 275 and 330 mμ. The curve of Gilman (1953) is similar to the acid spectrum (see also Sneath, 1956b).

No. 26. Bacille bleu IV Macé in Godfrin, 1934, pp. 72, 226. (Bacille bleu IV de Macé, 1898, cited by Godfrin, 1934, pp. 72, 226. ? Bacille bleu Macé, 1913, Vol. 2, p. 411).

Godfrin gives a good description of this organism but does not give the reference to Macé's paper, which I have not been able to find. The organism was isolated from water, and was a nonmotile, nonsporing, Gram-variable rod arranged in irregular clumps. It was pleomorphic and rather large, and showed Gram-positive granules at each end. Filamentous forms were common in old cultures. It grew well at 35° and at room temperature and gave abundant pigment at either temperature on suitable media. It liquefied gelatin very slowly, produced late acidity in milk and was indole-negative and produced no acidity on glucose agar.

On plain nutrient agar the growth was sometimes viscous and it produced very little pigment, but on agar containing glycerol, dextrin or maltose it produced a diffusing blue pigment. On gelatin it produced a violet pigment which Godfrin said was indistinguishable from the pigment of Bacillus violaceus, but which is not further described; this seems to be unlikely. It gave an olive coloured growth on potato. The blue pigment in liquid culture became reduced to a yellow pigment in the depths, and on shaking it became blue again. The pigment was apparently soluble in water.

This organism was probably similar to Chromobacterium viscosum Grimes, though the propertoes of the pigment are poorly described. Sneath (1956a) grouped it tentatively with the "indigo bacteria" but this is probably incorrect.

III (b). Species of Pseudomonas. The following bacteria seem with fair certainty to belong to the genus Pseudomonas.

No. 27. Pseudomonas aeruginosa (Schroeter, 1872, pp. 122, 126) Migula, 1900, p. 884.
(Bacterium aeruginosum Schroeter, 1872, pp. 122, 126. Pseudomonas aerugino a Migula, 1900, p. 884. Bacillus aeruginosus Schroeter, 1886, p. 157. Cromobacterium aeruginosum Bergonzini, 1879, p. 40. Bacillus pyocyaneus Gessard, 1882, p. 536; Godfrin, 1934, pp. 106, 239; Pseudomonas aeruginosa Breed et al., 1948, p. 89; Haynes in Breed et al., 1955, p. 99. Pseudomonas pyocyanea Wilson and Miles, 1955, Vol. 1, p. 595). Latin adj., like verdigris.

The full synonymy of this organism is not cited, nor the many names of bacteria which are probably varieties of it. The generic name Pseudomonas has been conserved with this species as type species (Judicial Commission, 1952). It has long been known that some strains of this organism produce black, blue and red colours on some media in addition to the typical diffusing blue-green and greenish-yellow pigments pyocyanin and fluorescin (Charrin and de Nittis, 1898). Pyocyanin is a blue pigment and is mentioned in Chapter X. Both are soluble in water and alcohol, but pyocyanin is soluble in chloroform while fluorescin (pyoverdin) is not.

The organism is well known to medical bacteriologists, since it is the cause of blue pus and is well described by Wilson and Miles (1955, Vol.1, p.595 et seq.). It is a straight rod, Gram-negative, nonsporing, motile by a single polar flagellum or less commonly by a tuft of polar flagella. It is strongly but not strictly aerobic, and although it oxidizes many carbohydrates it does not ferment them anaerobically (see Hugh and Leifson, 1953). It reduces nitrates, destroys nitrites, is strongly proteolytic and utilizes citrate for growth. There is no clear division between the strains isolated from diseases of animals, of plants, and from water and similar sources. A large number of variants differing in their pigment production have been described. There does not appear to be any report of a strain which produces a pigment which could plausibly be violacein. The name is validly published.

No. 28. Bacillus caryocyaneus Dupaix, 1930 ex Beijerinck cited by Dupaix, 1933, p.13.
(Bacillus caryocyaneus, Dupaix, 1933, p.13 et seq., Planche A, III, Fig.1; Godfrin, 1934, pp.130, 246. Bacterium caryocyaneum Dupaix, 1933, p.246. Pseudomonas caryocyaneus [sic] Beijerinck in lit. Dupaix, 1933, p.13. Pseudomonas caryocyanea Breed et al., 1948, p.146). Latin caryo-, nut or kernel, cyaneus, blue.

Most of the information on this bacterium is in the monograph of Dupaix (1933). It was isolated by Beijerinck from rotten wood and sent to the National Collection of Type Cultures, who sent it to Dupaix. Beijerinck isolated similar strains from "must" of a yeast manufactory and a brewery. It was a Gram-negative rod with a single polar flagellum, nonsporing and strictly aerobic. It liquefied gelatin and reduced nitrates to nitrites, and was indole-negative. On nutrient agar and gelatin it produced a fluorescent green pigment. On media containing glycerol and some other carbohydrates, on potato and in milk, it produced a blue pigment, caryocyanine (described more fully in Chapter X). It was slightly soluble in water. For its production oxygen, iron, magnesium, and phosphate are essential. The organism was not pathogenic for warm-blooded animals or for plants, but was virulent to larvae of the bee moth (Galleria mellonella). It cross-agglutinated weakly with Pseudomonas chlororaphis and with two fluorescent pseudomonads, and Dupaix considered it to be closely related to them. Unfortunately this strain appears to have been lost.

No. 29. Pseudomonas cattleyaecolor Marchal and Lotz, 1953, p.37.
Latin cattleya, generic name of a brilliantly colored orchid, color.

The description of this organism is very brief and therefore the name may not be validly published. It produces a violet water-soluble pigment which contains a pyrrolic nucleus and also iron; on removing the iron by chelating agents a colourless compound results (which is also formed if the organism is grown in media deficient in iron) which becomes violet on adding ferrous salts (Marchal, Clairet and Dupaix-Lasseur, 1953; Marchal and Clairet, 1953; Marchal and Dupaix-Lasseur, 1956a; Marchal and Bené, 1956). Magnesium is essential for the production of both the

pigment and its leucoderivative (Marchal and Dupaix-Lasseur, 1956b). Garibaldi and Neilands (1956) have recently reported similar coloured iron compounds which are produced by some bacteria. That produced by Bacillus subtilis is the ferric complex of 2, 3 dihydroxybenzoyl glycine (Ito and Neilands, 1958. See also No. 7 and No. 46).

No. 30. Bacillus cyaneofluorescens Zangemeister, 1895, p. 321. (Bacillus cyaneo-fluorescens Miquel and Cambier, 1902, p. 693; Godfrin, 1934, pp. 121, 243. Bacillus cyaneofluorescens Breed et al., 1948, p. 145. Pseudomonas cyaneo-fluorescens Migula, 1900, p. 906). Latin, blue and fluorescent.

This polar-flagellated organism was also from blue milk, but differed in several respects from Pseudomonas syncyanea (No. 33). It did not liquefy gelatin and produced on ordinary media a greenish-yellow fluorescent pigment. The growth on potato was brownish violet or flesh-coloured. The medium beneath colonies on gelatin was stained brownish violet. In unsterilized milk it produced a blue pigment which was insoluble in water, alcohol, or chloroform, but soluble in strong sulfuric acid giving a purple solution. It became red with caustic soda. Wolff (1913) also studied it. Lasseur, Marchal, Dupaix and Renaux (1932) studied a strain from the Král collection and made similar observations, but found it to be Gram-positive and to smell of trimethylamine. Their strain agglutinated with an antiserum against Bacillus Le Monnieri (No. 31) (quoted by Godfrin, 1934, p. 204). The name is validly published.

No. 31. Bacillus Le Monnieri Lasseur, 1913, p. 47. (Bacillus Le Monnieri Godfrin, 1934, pp. 124, 244. Bacillus lemonnieri Buchanan, 1936, p. 429. Flavobacterium lasseuri Bergey et al., 1930, p. 144. Pseudomonas lemonnieri Breed et al., 1948, p. 178; Hugo and Turner, 1957, p. 154. Pseudomonas Le Monnieri Villecourt and Blachère, 1957, p. 119, Figs. 1-3). Named for Prof. G. Le Monnier, a French scientist.

This was a nonsporing Gram-negative rod with a single polar flagellum. It liquefied gelatin. It produced a nondiffusing blue pigment on glucose media (not on media without carbohydrate) and also a yellowish fluorescent diffusing pigment. On potato it also produced reddish and violet diffusing pigments (Lasseur, 1913). It seems to have been a species of Pseudomonas since it cross-agglutinated with Pseudomonas syncyanea (No. 33) which in turn cross-agglutinated with Pseudomonas aeruginosa (No. 27) (Lasseur and Jouffroy, 1928).

The blue pigment was not identical with indigoidine from the "indigo bacteria" (Nos. 34-39) since the absorption spectrum in pyridine showed a maximum at 630 mμ (Lasseur and Girardet, 1926) and not at 605 mμ. Godfrin (1934, pp. 124-129) adds that it was strictly aerobic, indole-negative, reduced nitrate to nitrite and to gaseous nitrogen and dissociated into colonies which only gave the yellowish pigment (citing Lasseur and Vernier, 1924). Lasseur's strain has unfortunately been lost (J.G. Marchal, personal communication) but Hugo and Turner (1955, 1957) have isolated a bacterium from soil which appears to be the same organism,

and their description is in good agreement with that of Lasseur. Their strain is a Gram-negative rod with a single polar flagellum, strongly aerobic, growing between 2° and 30° and able to utilize citrate or gluconate as sole source of carbon and nitrate or ammonia as sole source of nitrogen. It reduced nitrates, destroyed nitrites, was indole-negative, V.P.-negative, H_2S-negative, NH_3-positive, catalase-positive. It liquefied gelatin and produced acid without gas from several carbohydrates, but only aerobically. The fluorescent pigment was indistinguishable spectroscopically from the green fluorescent pigment ('fluorescein' or pyoverdin, see Elliott, 1958) of Pseudomonas aeruginosa. The insoluble blue pigment was only formed upon media containing peptone and a carbohydrate, and its production was inhibited by the presence of sodium chloride. It is insoluble in water, alcohol, chloroform and light petroleum. It is soluble in dimethylformamide, in which it shows absorption peaks at 433 and 625 mμ and a transmittance peak near 500 mμ: the spectrum was different from that of indigoidine. They did not observe crystals of the pigment within the colonies as Lasseur did: however, Godfrin (1934, p. 186) mentioned that these crystals may have been mineral crystals stained blue by the pigment. Villecourt and Blachère (1957), have also studied this bacterium. They found it common in soil and to be readily isolated upon plates of silica-gel containing glucose and urea, on which after some days at 20° deep blue colonies form. They found that the nondiffusing blue pigment had similar properties to those found by Lasseur and Girardet (absorption maximum at 620 mμ and a shoulder at 580 mμ in pyridine). The bacteria also produced a green fluorescent diffusing pigment. They found their strains to be usually encapsulated as well as motile, but otherwise very similar to those of Hugo and Turner.

No. 32. Pseudomonas mildenbergii Bergey et al., 1930, p. 172. (Pseudomonas mildenbergii Bergey et al., 1934, p. 185; 1939, p. 134; Breed et al., 1948, p. 96; Haynes in Breed et al., 1957, p. 109. Pseudomonas cyanogena Bergey et al., 1923, p. 129, 1926, p. 134, not Bacillus cyanogenus Flügge, 1886, p. 291 (see No. 33). Blaubacillus Mildenberg, 1922, p. 309. Bacille bleu de Mildenberg, Godfrin, 1934, pp. 138, 248). Named for H. Mildenberg.

This organism was from air. It was a nonsporing Gram-negative rod with a polar flagellum. It slowly liquefied gelatin, grew slowly at 37°, gave a blue growth on potato, and on neutral gelatin medium containing glycerol it produced a blue pigment, but on acid media or plain gelatin a greenish pigment. Sterile milk became blue. The blue pigment was water-soluble, insoluble in chloroform, became red with acids and green with alkalis. It was probably a species of Pseudomonas, but may conceivably be related to Chromobacterium viscosum (No. 25).

No. 33. Pseudomonas syncyanea (Ehrenberg, 1840, p. 202) Migula, 1895, p. 29.
(Vibrio syncyaneus Ehrenberg, 1840, p. 202. Pseudomonas syncyanea Migula in Engler and Prantl, 1895, p. 29; Migula, 1900, p. 904; Holland, 1920, p. 224; Breed et al., 1948, p. 92; Haynes in

Breed et al., 1957, p.106. Bacterium syncyanum [sic] Schroeter 1872, p.126. Bacillus syncyanus Schroeter, 1886, p.157; de Toni and Trevisan in Saccardo, 1889, p.979; Macé, 1889, p.520; 1898, p.54 and Pl.27. Bacillus syncyaneus Bergey et al., 1923, p.288; 1926, p.295; Godfrin, 1934, pp.115, 242. Bacterium syncyanum Winter in Rabenhorst, 1881, p.53. Bacillus lactis cyanogenus Eisenberg, 1891, p.149. Vibrio cyanogenes Fuchs, 1841, p.190 quoted by Breed et al., 1948, p.92. Bacillus cyanogenus Trevisan 1889, p.18; Sternberg, 1893, p.636; Flügge, 1886, p.291; Matzuschita, 1902, p.358, Bacterium cyanogenus [sic] Chester, 1897, p.122. Cromobacterium [sic] syncyanum Bergonzini, 1879, p.39. ? Pseudomonas cyanogenes [sic] Holland, 1920, p.224. not Pseudomonas cyanogena Bergey et al., 1923, p.129, see No.32). From Greek, together, blue.

This is the bacillus of blue milk, and it is closely related to Pseudomonas aeruginosa (No.27), e.g. they show cross-agglutination (Lasseur and Jouffroy, 1928). It produces in milk, but not in other media as a rule, a water-soluble pigment which is blue when acidified as occurs when the lactic acid bacteria in milk begin to sour it (see Neelsen, 1880; Scholl, 1889; Godfrin, 1934, p.118). The name is validly published.

III (c). The "indigo bacteria."

The term indigo bacteria, though a misnomer since they do not produce indigo, is a useful one for a group of polar-flagellated rods which produce a very insoluble blue pigment (indigoidine). They probably belong to the genus Pseudomonas as was shown by the comprehensive study of Elazari-Volcani (1939) who is the authority upon them, but are treated separately here for convenience. It is possible that Bacillus Le Monnieri should be included among them, but the differences between the pigments are definite though slight. It should be noted that several very diverse bacteria produce the same blue pigment indigoidine (as shown by Starr, 1958, and see Nos.43, 51b, and 69a) so the group may be taxonomically heterogeneous.

These bacteria have seldom been isolated; most of the records are of isolations from water (see Macé, 1913, p.411). Very few workers have studied them themselves and some have confused them with chromobacteria. However, van Iterson (1903) had noticed that Bacillus indigoferus was commonly obtained by culturing soil in 2% calcium citrate with 0.2% potassium nitrate at 37° in the absence of air. Elazari-Volcani (1939) isolated a number of strains by the following technique. Glass bottles of 60 ml capacity with ground glass stoppers were inoculated with soil, mud, or water and filled to the top with a solution consisting of 2% calcium citrate (which of the salts is not stated), 0.2% potassium nitrate and 0.05% dipotassium hydrogen phosphate, stoppered and then incubated at room temperature or at 37°. When gas production ceased the culture was plated onto peptone agar and incubated at 20°. Occasionally a few blue colonies appeared, but by subculturing the fluid medium into a second bottle of the same medium these blue colonies could be regularly

obtained on plating the second liquid culture. They were isolated from most samples of soil and canal mud and from many samples of fresh water.

Elazari-Volcani gives good evidence that his isolates were the same bacteria as those of Claessen, Voges and other early workers. Elazari-Volcani observed two forms, one being motile and producing pigment in liquid media which he identified with Pseudomonas indigofera Voges (No. 37) and the other being nonmotile and not producing pigment in liquid media, which he considered to be a variety, Pseudomonas indigofera var. immobilis. The pigment has been described earlier (see Chapter X).

No. 34. Bacillus berolinensis indicus van der Sleen, 1894, No. 58, Pl. XIX, Fig. 58.
Latinized, of Berlin, and Indian dye, indigo; indigo bacillus of Berlin.

This was isolated several times from water. It was a motile non-sporing rod, sometimes slightly curved, showing bipolar staining, which produced deep blue colonies on gelatin and agar. It grew but produced little pigment at 37°. Gelatin was not liquefied. The blue pigment was insoluble in the usual solvents but was soluble in strong hydrochloric acid. It was clearly very similar to Claessen's bacillus (No. 36) as van der Sleen noted. The name is an illegitimate trinomial.

No. 35. Bacillus coeruleus Smith, 1887, p. 758.
(Bacillus coeruleus Adametz, 1888, p. 59; Trevisan, 1889, p. 20; de Toni and Trevisan in Saccardo, 1889, p. 986; Lustig, 1890, p. 88; 1893, p. 74; Eisenberg, 1891, p. 74; Germano, 1892, p. 517; Sternberg, 1893, p. 635; Voges, 1893, p. 302; Wright, 1895, p. 451; Kruse in Flügge, 1896, Vol. 2, p. 312; Macé, 1897, p. 852; Matzuschita, 1902, p. 138; Godfrin, 1934, pp. 40, 216; not Bacillus coeruleus Eckstein, 1894, p. 14 (No. 52). Bacillus Coeruleus Horrocks, 1901, p. 69 in part. Bacterium coeruleum Migula, 1900, p. 491. Bacterium coeruleus [sic] Chester, 1897, p. 118. Bacillus coerulens [sic] Calderini, 1925, p. 772. Chromobacterium coeruleum Ford, 1927, p. 475, not Chromobacterium coeruleum Bergey et al., 1923, p. 120, (No. 48). Bacillus caeruleus Macé, 1889, p. 525; Roux, 1892, p. 298; Frankland and Frankland, 1894, p. 474; Miquel and Cambier, 1902, p. 689 in part; Thiry, 1900, p. 103; Macé, 1913, p. 411; not Bacillus caeruleus Voges, 1893, p. 303 (No. 48), not Bacillus caeruleus Beijerinck, 1900, p. 6 (No. 49). Pseudomonas Smithii Chester, 1901, p. 318. Chromobacterium smithii Bergey et al., 1923, p. 121; 1926, p. 126, 1930, p. 160; 1934, p. 172; 1939, p. 94; Breed et al., 1948, p. 234. ? Bacterium caeruleum Lehmann and Neumann, 1899, Vol. 2, p. 265). From Latin, adj., sky-blue.

This organism was isolated by Smith from water of the River Schuylkill. It was a rod-shaped organism, often forming long chains, and sometimes the rods were curved. It gave a deep blue pigment on potato and gelatin, It liquefied gelatin and appears to have been facultatively

anaerobic. The pigment was insoluble in water and alcohol and was unchanged by acids. The name is validly published. Wright (1895) described a strain which he considered to be Smith's species, but his description differs in some respects. It is described separately (see No. 53).

No. 36. Bacillus indigoferus Zimmermann, 1893, p. 92. (Indigoblauen Farbstoff erzeugenden Bacillus, Claessen, 1890, p. 13. Bacillus indigonaceus Schneider, 1894, p. 228; Kruse in Flügge, 1896, Vol. 2, p. 314; Thiry, 1900, p. 104, Pl. IV, Figs. 2, 3; Matzuschita, 1902, p. 356; Calderini, 1925, p. 770; Godfrin, 1934, pp. 47, 218. Bacterium indigonaceum Lehmann and Neumann, 1896, Vol. 2, p. 267; 1899, Vol. 2, p. 264; 1920, Vol. 2, p. 405; 1927, Vol. 2, p. 465; Chester, 1901, p. 180. Bacillus Indigonaceus Horrocks, 1901, p. 70. Bacterium indigonaceus [sic] Chester, 1897, p. 118. Pseudomonas indigonacea Krassilnikov, 1949, p. 389. Bacillus berolinensis indicus Eisenberg, 1891, p. 114; Roux, 1892, p. 338; Germano, 1892, p. 517; Voges, 1893, p. 302; Frankland and Frankland, 1894, p. 473; van der Sleen, 1894, No. 58, Pl. XIX, Fig. 58. Bacillus berolinensis Indicus Sternberg, 1893, p. 621. Bacterium beriolinensis [sic] Indicus [sic] Chester, 1897, p. 118. Bacillus Beroliensis [sic] indicus Miquel and Cambier, 1902, p. 694. Pseudomonas berolinensis Migula in Engler and Prantl, 1895, p. 29; Migula, 1900, p. 948; 1901, p. 390; Bergey et al., 1939, p. 95; Breed et al., 1948, p. 697; Krassilnikov, 1949, p. 388. Pseudomonas Berolinensis Chester, 1901, p. 319. Bacillus berolinensis Macé, 1913, Vol. 2, p. 411, not Bacillus berolinensis Migula, 1900, p. 856. Bacterium berolinense Enderlein, 1925, p. 281. Bacille bleu-indigo, Roux, 1892, p. 338. Bacillo blue-indigo, Lustig, 1890, p. 78. Indigoblauer Bacillus, Lustig, 1893, p. 62. Pseudomonas indigofera Elazari-Volcani, 1939, p. 348). Modern Latin adj., indigo-bearing.

The synonymy of this organism is a rich mine of diversity of names and spellings, to which I trust I have not inadvertantly added. The organism was isolated by Claessen from water of the River Spree. It was a motile rod which produced on agar and on potato a deep indigo-blue nondiffusing pigment. It grew well at 20° and slightly at 37°; it did not liquefy gelatin. The pigment was studied in some detail by Schneider (1894) and was insoluble in water, alcohol, chloroform and carbon disulfide. It was soluble in strong acids: in hydrochloric acid it gave a blue solution, turning brown, in sulfuric and nitric acids it gave yellowish solutions. Potassium hydroxide turned it blue-green and ammonia decolorized it. Migula (1900, p. 948) found it to have a single polar flagellum, to produce a blue pellicle on broth and to give only slight growth in the depth of stab cultures so that it was evidently strongly aerobic. Thiry (1900, pp. 15, 78, 104, Pl. IV, Figs. 2, 3) studied the Král strain and noticed that the rods were sometimes curved, and its motility varied markedly with the conditions and the medium. He observed irregular blue or brownish masses of pigment within the colonies. The description of van der Sleen's strain (No. 34) agrees well with Claessen's.

Godfrin (1934, pp. 48-54) did not record the source of his strain, but said it was Gram-negative and that it sometimes possessed large intracellular granules. His strain produced a pigment which did not diffuse in agar but was stated to be soluble in water, chloroform and alcohol (presumably a misprint). It produced pigment best on potato when it was acid. It was inhibited by the presence of 3% glucose and did not produce acid from glucose. The pigment gave similar colour reactions to that of Claessen's strain. Lustig (1893, p. 62) studied a strain which was very similar to Claessen's. Zimmermann's name has priority to Schneider's. Zimmermann clearly indicated he was describing Claessen's bacillus. The name is validly published.

No. 37. Bacillus indigoferus Voges, 1893, p. 307.
(Bacillus indigoferus Kruse in Flügge, 1896, Vol. 2, p. 313; Macé, 1897, p. 853; 1913, Vol. 2, p. 412; Thiry, 1900, p. 104; Miquel and Cambier, 1900, p. 695; Matzuschita, 1902, p. 356; Calderini, 1925, p. 771; Godfrin, 1934, pp. 57, 221; Bergey et al., 1939, p. 95. Bacillus Indigoferus Horrocks, 1901, p. 70. Bacterium indigoferum Enderlein, 1925, p. 281. Bacterium indigoferus [sic] Chester, 1897, p. 118. Pseudomonas indigofera Migula, 1900, p. 950; 1901, p. 394; Chester, 1901, p. 319; Elazari-Volcani, 1939, p. 348; Figs. 1, 2; Krassilnikov, 1949, p. 388. Pseudomonas indigoferus [sic] Breed et al., 1948, p. 698). Modern Latin, indigo-bearing.

This organism isolated by Voges from water at Kiel, was a small Gram-negative rod, motile by a single polar flagellum. It produced on gelatin and most other media at room temperature a deep blue pigment which was insoluble in water, alcohol and fat solvents, but dissolved in strong acids and alkalis, giving colour reactions very similar to the pigment of Claessen's bacillus. The organism did not liquefy gelatin, gave no pigment at 37° and was not pathogenic on injection into a mouse. It gave a blue growth with a metallic sheen on potato. Voges thought it different from Claessen's bacillus (No. 36) but it is very similar, and Lehmann and Neumann (1899, Vol. 2, p. 264) and Elazari-Volcani (1939) considered them to be of the same species. It is uncertain whether Voges' use of the epithet "indigoferus" antedates Zimmermann's (see No. 36).

No. 38. Pseudomonas indigofera and var. immobilis Elazari-Volcani, 1939, pp. 348, 350, Figs. 1-3.
(Pseudomonas indigoferus [sic] and var. immobilis Breed et al., 1948, p. 698). Modern Latin, indigo bearing, and Latin, immobile.

These were isolated from soil and water by Elazari-Volcani. They were rods, about 0.5 x 1-2.5 μ, which if motile had a single polar flagellum. On peptone agar at 20° they formed circular smooth convex colonies, blue in the centre, and which after a week developed a coppery-red reflex. The agar beneath was stained blue, showing characteristic rings of pigmentation, and the colonies contained small granules of deep blue pigment. The growth on peptone agar slants was butyrous, opaque,

raised and glistening, pale yellowish, and later blue with a coppery reflex. In peptone water the organisms gave turbidity and a pellicle (often blue); on shaking the tube the faint blue fluid may become a deeper blue. Gelatin stab cultures showed a 'nail-head' growth, i.e. a button of blue growth on the surface and a colourless filiform growth below: no liquefaction occurred even after several months. In liquid media no acid or gas was produced from carbohydrates, although sucrose was hydrolyzed. Nitrates were reduced to nitrites but not to nitrogen gas. Growth occurred at 37° but was colourless. The indole reaction was strongly positive. Elazari-Volcani states that similar strains were isolated from the Dead Sea and by Hoogerheide in Holland. The blue pigment is only formed in the presence of abundant oxygen. He obtained it by growing the organism in sucrose peptone water in thin layers or in larger volume under forced aeration, and extracting the bacteria first with hot chloroform and hot alcohol to remove fats and then with hot pyridine. The pigment was soluble in pyridine but not in ordinary solvents such as water, alcohol, chloroform or ether. It was soluble in strong hydrochloric acid, giving a blue solution and in strong sulfuric acid, giving a brown solution. In potassium hydroxide it gave a blue solution which soon turned yellow. These observations are in close agreement with those of earlier authors. The pigment was not indigo (indigotin) and Elazari-Volcani called it indigoidine. It is more fully described in Chapter X. Elazari-Volcani has studied its biosynthesis but has not published the details (see Volcani, 1954). The organism has a remarkable ability to oxidize ortho-aminophenols to coloured compounds, and it can, for instance, oxidize 3-hydroxyanthranilic acid or 3-hydroxy kynurenine to a deep brown pigment similar to the ommachromes of the eye of Drosophila (Volcani, personal communication).

The variety immobilis is Elazari-Volcani's Type II. It differs from Type I in being nonmotile and producing less pigment, especially in liquid media. The names are validly published. Type I, however, should have been termed Pseudomonas indigofera var. indigofera.

No. 39. Bacillus pavoninus Forster in van der Sleen, 1894, No. 59. (Bacillus pavoninus Thiry, 1900, p. 104, Pl. IV, Fig. 4; Godfrin, 1934, pp. 46, 217; Breed et al., 1948, p. 233). From Latin pavo a peacock, peacock-soloured.

This organism was isolated by J. Forster from water and his description was published by van der Sleen who attributed the name to him. It was clearly very similar to Claessen's bacillus (No. 36) as van der Sleen pointed out, both organisms being motile nonsporing rods which did not liquefy gelatin and which formed blue colonies with a pale yellow edge.

Forster's bacillus grew well on potato giving a deep blue growth. It grew at 37° but showed little pigment at this temperature; pigmentation was best at room temperature. In broth it gave a fragile pellicle and the fluid became greenish blue. The indigo-blue pigment was insoluble in wzter, alcohol and chloroform, and became green on treating with alkalis. Forster believed that it could cause blue discoloration of Edam cheese. Thiry studied a strain and found it to be comma-shaped. The name is validly published.

III (d). Species of Micrococcus.

No. 40. Micrococcus moricolor Holmes and Wilson, 1945, p. 312. (Micrococcus moricolor Breed et al., 1948, p. 696). From Latin, morum a mulberry and color, colour. Modern Latin noun, mulberry-colour.

This seems to have been a typical micrococcus which produced a purple nondiffusing pigment but only on potato. The name is validly published.

No. 41. Micrococcus purpurifaciens Lehmann and Neumann, 1920, Vol. 2, p. 755.
(Micrococcus of Dudtschenko, 1914, Micrococcus purpurifaciens Lehmann and Neumann, 1927, Vol. 2, p. 296; Breed et al., 1948, p. 272. From Latin purpur, purple and facio, make. Modern Latin part., making purple.

This was a nonmotile Gram-positive encapsulated coccus from ice. It produced a purple diffusing pigment on alkaline gelatin. The name is validly published.

No. 42. Micrococcus violagabriellae Castellani, 1955, p. 477, Fls. I and II.
(Micrococcus violagabriellae, Stocchi, 1956a, p. 347; 1956b, p. 596). Etym. dubious.

This organism was isolated by Castellani from cases of axillary dermatosis, and he considered that it may have been the causative agent. It is a typical Gram-positive micrococcus which produces a violet nondiffusing pigment, best on potato and on glucose agar. He wondered if it was a variety of Micrococcus pyogenes, M. pyogenes var. violagabriellae. Stocchi (1956a; 1956b) has studied it and concluded that it was a new species, but his data are not convincing, since it was compared with only two other strains of micrococci. The name is validly published.

Professor Castellani kindly sent me a strain. On examination it was a typical Micrococcus, being a Gram-positive nonmotile sphere, arranged in clumps, catalase-positive, phosphatase-positive, weakly urease-positive, and giving acid from glucose, maltose, sucrose but not from mannitol, lactose or salicin in peptone water carbohydrates. It was V.P. positive and coagulase-negative (rabbit serum, tube test). On potato it gave a reddish diffusing pigment after a week at 20°, but very little on glucose agar. It would appear to belong to the subgroup 2 of Shaw, Stitt and Cowan (1951) which they call Staphylococcus saprophyticus and does not seem to be a distinct species.

III (e). Probably species of Corynebacterium.

No. 43. Aplanobacter insidiosum McCulloch, 1925, p. 497.
(Aplanobacter insidiosum Jones and McCulloch, 1926, p. 493.
Bacterium insidiosum Stapp in Sorauer, 1928, p. 178.

Corynebacterium insidiosum Jensen, 1934, p.41; Breed et al., 1948, p.392; Burkholder in Breed et al., 1957, p.591. Burkholderiella insidiosa Săvulescu, 1947. Phytomonas insidiosa Bergey et al., 1930, p. 278. Mycobacterium insidiosum Krassilnikov, 1941, p.102). Latin adj., insidious.

This organism causes a disease of alfalfa (Medicago sativa) and is clearly very closely related to certain other plant pathogens which are usually included in Corynebacterium; thus Rosenthal and Cox (1953; 1954) found that it had antigens in common with Corynebacterium michiganense (Smith) Jensen, 1934, p.47. It is a nonmotile Gram-positive encapsulated bacillus which produces yellow colonies within which are deep blue pigment granules. It slowly liquefies gelatin. The pigment does not diffuse. The name is validly published.

Its chief interest is that the pigment seems to be identical with indigoidine from Pseudomonas indigofera (No.38 and see Chapter X) and also with the pigments of Erwinia chrysanthemi (No.51b) and of a strain of Arthrobacter (Starr, 1955; 1958; Kuhn and Starr, 1956). These organisms are very different from one another.

No.43a. Mycobacterium caesium Krassilnikov, 1949, p.170, Tab.1, Fig.1. Latin adj., blue-grey.

An organism from soil, very similar to the last. It is a Gram-positive pleomorphic rod with metachromatic granules producing on ordinary media pale blue colonies. The pigment does not diffuse. Gelatin is very slowly liquefied; milk is peptonized or clotted. The name is validly published.

No.43b. Mycobacterium cyaneum Krassilnikov, 1941, p.100. (Corynebacterium insidiosum var. saprophyticum Jensen, 1934, p.42). Latin adj., blue, from Greek.

A strain isolated from soil and not pathogenic to lucerne. It was a Gram-positive pleomorphic rod very similar to No.43, and like it produced a blue insoluble pigment on nutrient agar containing glucose. Krassilnikov implies that the pigment diffuses, evidently an error. The name is validly published.

III (f). Purple variety of Pasteurella pestis.

No.44. Pasteurella pestis (Lehmann and Neumann, 1896, Vol.2, p.194) Holland, 1920, p.219.
(Pestbazillen, Pigmentbildende Variante, von Preisz, 1926, p.67).

The plague bacillus is normally not pigmented but von Preisz described a variant producing a black or violet-black pigment. Bessonowa and Lochow (1930) also reported a similar variant which produced a violet pigment. It would be tempting to identify these as chromobacteria whose identity was misdiagnosed, but the descriptions suggest that they were indeed plague bacilli.

III (g). Bacteria of uncertain genus.

There is a large number of blue and violet bacteria which are too poorly described to enable one to classify them. A few are described sufficiently for one to recognize them as distinct species but most of them are unrecognizable.

No.45. Bacterium anthocyaneum Reiss, 1912, p. 129.
From Greek, flower and blue, Modern Latin, flowery-blue.

This was reported as a motile Gram-positive rod producing a bluish pigment on potato and in milk. On gelatin the growth was yellowish, and it gave slow liquefaction. It is unrecognizable. The name is validly published.

No.45a. Bacillus azureus Matzuschita, 1902, p. 308.
(Not Bacillus azureus Zimmermann, 1893, p. 100).
Latin adj., sky-blue.

Matzuschita attributes this species to Zimmermann but Zimmermann's organism did not produce pigment (see No.10). Matzuschita describes a motile rod which gives on gelatin sky-blue colonies containing a blue pigment. Gelatin was not liquefied. On potato the growth was brown. It may have been an "indigo bacterium" (see Nos. 34-39), but is now unrecognizable. The name is a later homonym of Zimmermann's, but is validly published.

No.46. Bacillus bruntzii Nepveux, 1920a, cited by Melcion, 1945, pp. 23, 149.
(Bacillus bruntzii Nepveux, 1920b, p. 742; Breed et al., 1948, p. 644). Named for Professor Bruntz.

The original description (Nepveux, 1920a) of this organism has not been seen by me. Nepveux (1920b) and Melcion (1945, p. 23) described it as producing a violet pigment which was also fluorescent. The pigment contained iron, and in the absence of iron the organism produced a yellow substance which became deep reddish-violet on adding traces of iron salts. It cannot be recognized from the few details I have found. The behaviour with iron is similar to that shown by Pseudomonas cattleyaecolor (No. 29) and is very suggestive of the iron complex of 2,3 dihydroxybenzoylglycine (Ito and Neilands, 1958). Lasseur (cited by Breed et al., 1948, p. 645) considered that Bacillus bruntzii was identical with Bacillus roseus fluorescens of Marchal (see Marchal and Béné, 1956).

No.47. Bacillus caeruleo-violaceus Tager, 1893.
From Latin, sky-blue and violet.

I have not been able to consult the original description of this organism. It was said to produce a pigment similar to violacein. It is incerta sedis.

No. 48. Bacillus caeruleus Voges, 1893, p. 303. (Bacillus caeruleus Thiry, 1900, p. 103; Miquel and Cambier 1902, p. 689 in part; Macé, 1913, Vol. 2, p. 412, not Bacillus caeruleus Beijerinck, 1900, p. 6 (No. 49). Bacillus coeruleus Kruse in Flügge, 1896, Vol. 2, p. 313; Calderini, 1925, p. 772; Godfrin, 1934, pp. 40, 215, not Bacillus coeruleus Smith, 1887, p. 758 (No. 35) not Bacillus coeruleus Eckstein, 1894, p. 14 (No. 52). Bacillus Coeruleus Horrocks, 1901, p. 70 in part. Bacillus pseudocoeruleus Matzuschita, 1902, p. 140. Bacterium caeruleum Lehmann and Neumann, 1899, Vol. 2, p. 265; 1920, Vol. 2, p. 406; 1927, Vol. 2, p. 465. Bacterium coeruleus [sic] Chester, 1897, p. 117, not Bacterium coeruleus [sic] Chester, 1897, p. 118. Bacterium caerulea [sic] Enderlein, 1925, p. 281. Pseudomonas caerulea Migula, 1900, p. 945; 1901, p. 386; Krassilnikov, 1949, p. 389. Pseudomonas coerulea Chester, 1901, p. 318. Pseudomonas cerulea [sic] Holland, 1920, p. 217. Chromobacterium coeruleum Bergey et al., 1923, p. 120; 1926, p. 125; 1930, p. 160; 1934, p. 172; 1939, p. 94; Breed et al., 1948, p. 234. ? Chromobacterium coeruleum Topley and Wilson, 1929, Vol. 1, p. 402. Not Chromobacterium coeruleum Ford, 1927, p. 475 (No. 35). Latin adj., sky-blue.

This organism was a small Gram-negative rod measuring 0.7 to 0.9 by 0.9 to 1.4 μ. It was motile by a single polar flagellum. It was non-sporing and facultatively aerobic, and it slowly liquefied gelatin. It grew on potato. Milk was not coagulated. It grew at 37° and produced a little H_2S. On gelatin it produced a blue pigment which was said to be soluble in water and in alcohol but not in chloroform or benzene, although Voges does not make it clear whether this pigment diffused through the medium. Pigment granules were observed within the colonies.

The pigment became green with acetic acid, and remained blue when treated with ammonia. The organism was isolated from water at Keil. It was not pathogenic on injection into mice.

Other authors report that the properties of the pigment were different. Thus Lehmann and Neumann (1899, Vol. 2, p. 265) found it insoluble in all ordinary solvents. Rosenberg (1899, pp. 33, 35, quoted by Thiry, 1900, p. 80) found it not to diffuse in the medium and Godfrin (1934, pp. 43, 44) quotes other findings of Rosenberg's. However, it is uncertain whether these authors were studying Voges' strain, or other organisms such as Bacillus coeruleus Smith (No. 35). Voges regarded his organism as distinct from Smith's.

It is impossible now to be sure of the taxonomic position of Voges' bacillus. It is not a strain of Chromobacterium. Voges' name is an orthographic variant of Smith's name, and is thus a later homonym. The epithet "pseudocoeruleus" was validly published by Matzuschita.

No. 49. Bacillus caeruleus Beijerinck, 1900a, p. 6; 1900b, p. 31. Latin adj., sky-blue.

An organism named but not described: the name is therefore a nomen nudum and is not validly published.

No. 50. Chromobacterium chocolatum Lasseur et al., 1944b, p. 293, Pl. X figs. 1, 2, 25, ex Knutsen.
(Chromobacterium chocolatum Breed et al., 1948, p. 693; Gilman, 1953, p. 48). Modern Latin, chocolate-brown.

This organism was briefly mentioned but not described by Lasseur and Giabicani (1944, p. 164) and Lasseur et al., (1944a; 1945). It was named by Knutsen (of State College, Pennsylvania, USA) but apparently he did not publish the name. Lasseur et al., (1944b; 1944c) gave a brief description, mainly serological. Lasseur and his colleagues noted that it produced several pigments, notably a violet-brown and an orange pigment, and the culture gave rise to orange variants. They distinguished the latter as Chromobacterium orangium (No. 51).

Gilman (1953) made a full study of the organism which is American Type Culture Collection 7320. He found it to be a Gram-positive nonmotile rod, without a capsule and without spores. It produced on nutrient agar a brownish-orange growth, and produced acid without gas in glucose and most other carbohydrates tested. It was indole-negative, catalase-negative, and hydrolyzed starch. It grew at 37° but not at 4°. The pigment was apparently insoluble in water but soluble in alcohol, in which solvent it showed absorption peaks at 440 mμ, 470 mμ, 510 mμ, and 570 mμ, which was suggestive of a carotenoid pigment.

This organism is clearly not a strain of Chromobacterium, and at present its taxonomic position is uncertain. The descriptions of Lasseur and his colleagues and of Breed et al., are so brief that there is doubt on whether they validly published the name; if they did not, then Gilman's name is validly published.

No. 51. Chromobacterium orangium Lasseur et al., 1944b, p. 294.
(Chromobacterium chocolatum Breed et al., 1948, p. 694; Chromobacterium orangium Gilman, 1953, p. 48). Latinized, orange-coloured.

This is the orange variant of the last (American Type Culture Collection 7319), and Gilman confirmed this. The validity of the names is doubtful (see above), but taxonomically it must be regarded as a variety, not as a distinct species.

No. 51a. Cyanococcus chromospirans Färber, 1951.
Greek-Latin hybrid part., etym. dubious, probably coloured and respiring.

I have not seen the original description of this organism. It is reported to be a small nonmotile coccus, nonproteolytic, but with a smell of fresh glue. The optimum temperature for growth is about 27°. It rapidly oxidizes certain sugar acids to keto-acids. It produces a blue pigment which was identified as pyocyanin (see Chapter X and No. 27). It may prove to be recognizable, but at present it is of uncertain genus.

No. 51b. Erwinia chrysanthemi Burkholder, McFadden and Dimock, 1953, p. 526.
(Erwinia chrysanthemi Breed et al., 1957, p. 353). Latinized Greek, of the chrysanthemum plant.

This organism, which causes a blight of florists' chrysanthemums accompanied by soft rot, frequently produces the blue pigment indigoidine according to Starr (1958), the same pigment as that of the Pseudomonas indigofera group (Nos. 34-39) and of Corynebacterium insidiosum (No. 43). It is a Gram-negative nonsporing rod with peritrichous flagella, which liquefies gelatin, produces acid and gas from many carbohydrates and liquefies pectate media. Slants on nutrient agar may contain slate grey deposits of the pigment. It appears to be a member of the soft rot section of Erwinia. The name was validly published.

No. 52. Bacillus coeruleus Eckstein, 1894, p. 14.
(Bacillus coeruleus Breed et al., 1948, p. 652, not Bacillus coeruleus Smith, 1887, p. 758, not Bacillus caeruleus Voges, 1893, p. 303, not Bacillus caeruleus Beijerinck, 1900, p. 6, see Nos. 35, 48, 49). Latin adj., sky-blue.

This was from the larva of the nun moth (Liparis monacha) and was poorly described. It was a rod which liquefied gelatin very slowly and on potato produced a "blue-white" growth. Its pigmentation was very slight, and it is unrecognizable. The name is a later homonym of B. coeruleus Smith (No. 35), but is validly published.

No. 53. Bacillus coeruleus Wright, 1895, p. 451.
(Chromobacterium coeruleum Ford, 1927, p. 475).
Latin adj., sky-blue.

Wright considered that he had re-isolated Bacillus coeruleus Smith, (No. 35), but his description is not in complete agreement and is not any fuller than Smith's. It was a motile bacillus with several flagella which did not form spores. It slowly liquefied gelatin, producing a slatey blue growth. On agar and potato the growth was slate blue, and a blue ring formed on broth cultures. It grew at 20° and at 36°. The pigment was not described. The organism was from Schuylkill River water. This bacterium is incertae sedis. The name was validly published.

No. 54. Bacterium cristallino violaceum Cholkevitch, 1922, in Godfrin, 1934, pp. 92, 233.
(Bacterium cristallino violaceum Waeldele, 1938, p. 39; Breed et al., 1948, p. 234). Latin, crystalline and violet-coloured.

Godfrin does not give the reference to Cholkevitch in his bibliography. Godfrin's description is of a motile Gram-negative slender rod, pleomorphic in old cultures, and containing metachromatic granules. It possessed a capsule but did not form spores. It was said to contain volutin granules. In culture on the usual media it formed a violet pigment, which could be seen as needle-shaped crystals in fan shaped clusters, which were violet, reddish or even yellow in colour. The pigment was insoluble in water, alcohol, chloroform and other common solvents. but dissolved in dilute alkalis to give a bluish solution which on acidification became violet and was stable.

The crystals showed two spectral absorption bands, one from 585 to

625 mμ and a fainter one from 480 to 510 mμ (Lubimenko, in Godfrin, 1934, p. 94.

The organism was from peat near Leningrad. It was clearly not a strain of Chromobacterium and seems unrecognizable, though the pigment was evidently not unlike indigoidine. The name is validly published by Godfrin, as it is not a trinomial.

No. 55. Bacillus cyaneo-fuscus Beijerinck, 1891a, para 706 and Pl. 1; 1891b, p. 308; 1892, p. 228 and Pl. 1. (Bacillus cyaneo-fuscus Macé, 1897, p. 849; 1901, p. 911; Thiry, 1900, p. 102; Godfrin, 1934, pp. 67, 225; Breed et al., 1948, p. 233. Bacillus cyano-fuscus [sic] Sternberg, 1893, p. 727. Bacterium cyano fuscus [sic] Chester, 1897, p. 116). From Greek, Latinized adj., blue and Latin adj., dark-coloured.

Beijerinck found this organism several times, and isolated it from blue spots on cheese, on flour paste and also from water and soil by using a whey and peptone medium. It was a slender motile rod, which grew only at low temperatures (6 to 10°). It was strictly aerobic. It produced a blue pigment which turned brown, and which was found in granules outside the cells. The pigment was slightly soluble in water, and was soluble in strong acids. Its systematic position seems obscure. Beijerinck's name is validly published.

No. 56. Bacteridium cyaneum Schroeter, 1872, pp. 122, 126. (Micrococcus cyaneus Cohn, 1872, p. 156 in part. Micrococcus cyaneus Winter in Rabenhorst, 1881, p. 44; Grove, 1884, p. 9; Schroeter, 1886, p. 145; Trevisan, 1889, p. 34; Macé, 1889, p. 356; 1897, p. 437; 1901, p. 438; Lustig, 1890, p. 60; 1893, p. 35; Roux, 1892, p. 286; Bergonzini, 1892, p. 200; Viron, 1892, p. 180; Frankland and Frankland, 1894, p. 495; Lehmann and Neumann, 1896, Vol. 2, p. 179; 1899, Vol. 2, p. 179; 1920, Vol. 2, p. 256; 1927, Vol. 2, p. 787; Thiry, 1900, p. 98; Migula, 1900, p. 187; 1901, p. 127; Chester, 1901, p. 109; Calderini, 1925, p. 773; Godfrin, 1934, pp. 29, 212; Breed et al., 1948, p. 259. Mikrococcus cyaneus Adametz, 1888, p. 35; Matzuschita, 1902, p. 554. Cromococcus [sic] cyaneus Bergonzini, 1879, p. 37. Nigrococcus cyaneus Castellani and Chalmers, 1919, p. 932. ? Blauer coccus Maschek, 1887, p. 67. Not Micrococcus cyaneus (Bacterium cyaneus [sic] White, 1906, p. 16 (No. 11). Not Bacterium cyaneum Leonard, 1904, p. 398 (No. 21). Latin adj., dark blue, from Greek.

There is a good deal of confusion about this organism, since the original description is brief and a number of other authors who believed they had re-isolated it gave conflicting descriptions (Adametz, 1888; Maschek, 1887, see No. 72; Viron, 1892). Schroeter described a non-motile coccobacillus found on potato exposed to the air in a mixed culture with a fungus. It produced deep blue colonies. The pigment was water-soluble, diffused through the potato and became carmine red when acidified and deep blue when alkaline. Cohn found it grew at 30° in an ammonium acetate tartarate medium. Beijerinck (1913) considered it was

close to the actinomyces and Müller (1908) compared it with Bacterium coelicolor (No. 19). The name is validly published.

No. 57. Actinococcus cyaneus Beijerinck, 1913, p. 198, Figs. 1-4. (Actinococcus cyaneus Breed et al., 1948, p. 923. Proactinomyces cyaneus Krassilnikov, 1938, p. 164 quoted by Breed et al., 1948, p. 923). Latin, dark blue, from Greek.

This organism was discovered by Beijerinck in soil. It was a coccobacillus, sometimes showing thread-like forms and perhaps rudimentary mycelium. It produced a blue pigment in culture. It slowly liquefied gelatin. The water-soluble blue pigment became red in acids and blue with alkalis.

Beijerinck considered this organism to be close to Schroeter's Bacteridium cyaneum (No. 56). Gause described an organism which he considered similar to Beijerinck's bacterium (Proactinomyces cyaneus-antibioticus Gause, 1946, p. 649) and which was clearly an actinomycete forming a definite mycelium. Its pigment also became red with acids and blue with alkalis.

The systematic position of Beijerinck's organism seems rather uncertain, so it is listed here as incertae sedis. The name appears to be validly published.

No. 58. Micrococcus cyanogenus Pammel and Combs, 1896, p. 136. (Micrococcus cyanogenus Breed et al., 1948, p. 259). From Greek, blue producing.

Details of this organism are very scanty, and it seems unrecognizable. It turned milk blue. It was a coccus which liquefied gelatin but produced no pigment on this medium. The name is validly published.

No. 59. Chromobacterium ianthinum Gilman, 1953, p. 48. (Not Chromobacterium ianthinum Breed et al., 1948, p. 232. Nos. 115, 117), not Chromobacterium janthinum Holland, 1920, p. 222 (No. 115). (pro synon. Bacterium ianthinum Zopf, 1883, p. 68). Strain S11 of an unnamed pseudomonad, Morris and Roberts, 1959.) From Greek, violet-coloured, Latinized.

This organism is the strain studied here as Strain TI. It was isolated from forest soil in Trinidad and mislabelled Chromobacterium ianthinum (Morris, personal communication). Gilman accepted the name on the label, but it is clearly not the Chromobacterium ianthinum of other authors. Gilman found it to be a Gram-negative motile rod which was catalase-negative and did not reduce nitrate to nitrite. The pigment was reddish and was said to be insoluble in water but soluble in alcohol, and Gilman found small absorption peaks at 490 mμ, 410 mμ and 320 mμ.

I have found the pigment to be of two sorts, which may be chemically related forms, a yellow pigment, not diffusing, which is evidently the form giving Gilman's absorption spectrum, and a magenta diffusing pigment formed only on media containing carbohydrate. The organism is a polar flagellate (lophotrichous) Gram-negative rod, most probably

a pseudomonad (Sneath, 1956b), and is clearly not a strain of Chromobacterium. The name is an illegitimate later homonym of Chromobacterium janthinum Holland. I do not know the systematic position of this strain, and have not given it a name (see Sneath, 1957b).

Morris and Roberts (1959) have recently studied this group of bacteria, isolated from soil in Trinidad, and consider it to belong to Pseudomonas, though they do not give it a specific epithet. Their findings are in close agreement with my own, and strain TI seems to be a typical strain of the group. Morris and Roberts divide the group into two: strains of one produce a purple pigment and do not reduce nitrate, strains of the other subgroup produce only yellow pigment and do reduce nitrate. Strain TI belongs to the former subgroup. Morris and Roberts found that these strains produce a diffusable yellowish pigment which fluoresces violet in ultraviolet light (not green like the fluorescent pigment of Pseudomonas aeruginosa) when grown on asparagine and salts medium.

Description of Strain TI. The methods are those used for Chromobacterium and details are given in Appendix I.

Morphology. A Gram-negative rod, about 0.9 x 2.5 μ, one to three long flagella arising from one pole, showing bipolar staining, fat droplets, but no spores, capsule, or metachromatic granules. Morris and Roberts found that the fatty material was poly-β-hydroxy butyric acid, and formed about 30% of the dry weight of the cells (see also Chapter XV). Hayward, Forsyth and Roberts (1959) found that it could later be metabolized.

Cultural behaviour. Colonies on nutrient agar are low convex, smooth, shiny, round with entire edge about 0.5 mm in diameter after 1 day at 25°. They were pale yellow semitransparent and slightly viscid. There is little or no haemolysis on blood agar. Nutrient agar slant (2 days, 25°): a pale yellow shiny growth with lobate edge. Gelatin stab (7 days 20°): moderate napiform liquefaction with filiform growth in the stab and yellow pellicle. Nutrient broth: moderate turbidity, a viscid yellow ring of growth at the surface, a thin pellicle and some viscid deposit. Löffler's serum is digested in 7 days. On potato a smooth glistening growth forms which after 4 days at 25° became red-violet, later brown-red, the pigment diffusing slightly.

Resistance. The resistance to heat and phenol is that usual in vegetative bacteria: it is penicillin-resistant.

Metabolism and nutrition. It is fairly strictly aerobic, grows from 10° to 37° (optimum c. 30°), grows from pH 6 to pH 9, grows on 3.5% NaCl agar but not on 6.5%, produces no HCN and grows in Koser's citrate.

Carbohydrates. It produces acid (but no gas and only aerobically) in Hugh and Leifson medium from glucose, fructose, mannose, L(+) arabinose, maltose, sorbitol, galactose, lactose, cellobiose, and m-inositol, late acid from trehalose, salicin and mannitol, doubtful acid from D(+) xylose, no acid from inulin, sucrose, glycerol, or starch. In peptone water media it gives acid only from glucose.

Biochemical. Litmus milk: slight or doubtful acid, clot slight or absent. Indole -, NH_3 +, MR -, VP -, MB reduction weak, H_2S -, Nitrate reduction -, nitrite destruction -, catalase +, urease -, phosphatase

strong +, aesculin hydrolysis +, casein hydrolysis weak, gelatin hydrolysis strong, starch hydrolysis -, haemolysis weak, egg-yolk reaction +, malonate -, phenylpyruvate -, gluconate test +, arylsulfatase -, chitin digestion -. Morris and Roberts (1959) report it as strongly lipolytic but not cellulolytic.

Pigment. On nutrient agar, peptone agar with 0.5% NaCl and the latter plus 1% glucose it gives only a pale yellow pigment which diffuses slightly. On potato and on peptone agar with 0.5% of salt plus 1% sucrose or 1% mannitol it gives a poorly diffusing magenta pigment at 20° or 25° but little at 37°. In old peptone water carbohydrate tubes a dark red pigment may form in the pellicle which is almost insoluble in water, ethanol, chloroform or benzene, but which dissolves in 0.1 M HCl and becomes yellow: it can then be extracted into chloroform. On shaking the chloroform with 0.1 M NaOH it becomes red-violet and passes into the aqueous phase. The three pigments may be closely related, since the yellow one becomes mauve with alkali and the mauve one yellow with acid. The mauve pigment from mannitol peptone agar cultures was extracted with chloroform after scraping the growth into 0.1 M HCl, and the yellow chloroform was extracted with 0.1 M NaOH giving a mauve solution. The aqueous phase was then reacidified with HCl and the process repeated twice. This partly purified pigment was yellow when extracted into chloroform from acid and was stable for some hours: the absorption spectrum showed maxima at 277, 367 and 447 mμ and minima at 307 and 406 mμ. When extracted into 0.1 M NaOH the unstable mauve solution had maxima at 255, 285 and 536 mμ and minima at 262 and 446 mμ with shoulders at about 360 and 400 mμ (see Fig. 11 and Sneath, 1956b). This pigment is clearly not violacein. Morris and Roberts (1959) purified the insoluble dark-red pigment of another strain of this group (strain B175) by chromatography of the acid chloroform solution upon silica gel. It was recrystallized from benzene-cyclohexane and gave orange-yellow needles M. Pt. 237°-238°, of empirical formula $C_{16}H_{12}N_2O_6$ containing one methoxy group and one N-methyl group. On hydrolysis with alkali it yielded a red acid, M. Pt. 284°, which gave phenazine on distillation with zinc dust. This pigment is, therefore, clearly a phenazine derivative.

No. 60. Halobacter innocens violet variety Anderson, 1954, p. 267.
Latin adj., harmless.

Anderson reported that one strain of halophilic bacteria produced a violet pigment. His other strains (which were from salted hides and salted fish) were red chromogens, and he considered that the violet strain (strain NB 8) was a variety of Halobacter innocens, which belongs to the group of red Gram-negative obligate halophils which do not ferment carbohydrates. The systematic position of the genus Halobacter is still uncertain: it does not seem to be closely related to Chromobacterium. The name is validly published.

No. 61. Chromobacterium iodinum Davis, 1939, p. 273.
(Chromobacterium iodinum Clemo and McIlwain, 1938, p. 479;

Breed et al., 1948, p. 694; Gilman, 1953, p. 48. Pseudomonas iodinum Tobie, 1939, p.16; 1940b, p.1036; Haynes in Breed et al., 1957, p.106. Pseudomonas iodina [sic] Tobie, 1945, p. 460. Pseudomonas clemoi Tobie, 1939, p.16. Pseudomonas clemo [sic] Tobie, 1940a, para. 1350; 1940b, p.1036; 1945, p.460). Modern Latin noun, iodine.

This organism was isolated by Davis from milk. He described it as a Gram-negative nonsporing nonmotile bacillus which produced on peptone agar a deep purple pigment which appeared within the colonies as small crystals with a metallic sheen. The organism was facultatively anaerobic, indole-negative, catalase-positive, reduced nitrate to nitrite, liquefied gelatin and produced alkalinity in milk.

The pigment (iodinin) is insoluble in water and in cold alcohol and is very soluble in chloroform and other fat solvents. It is 1:6 dihydroxyphenazine 5:10 dioxide (see Chapter X). Gilman (1953) studied the organism, but found it to be Gram-positive. He found that it was "reductase positive" and utilized citrate for growth. The pigment which he studied was described as brownish and diffusing, and as having in aqueous solution absorption peaks at 350 mμ and 540 mμ and transmittance peaks at 320 mμ and 440 mμ, which is not in agreement with the properties of iodinin, but his figure suggests a solution in chloroform.

Tobie (1939; 1940a) suggested that the organism should be transferred to the genus Pseudomonas because its pigment (which he called iodinine or clemoine) was a phenazine derivative. Gilman concurs in this. I do not agree that the organism should be included in Pseudomonas, since it is Gram-positive with the morphology of a diphtheroid: it may belong to the genus Corynebacterium or to the genus Brevibacterium Breed (1953, p.13) which Breed established for Gram-positive nonsporing rods with poor fermentative powers. It is quite clearly not a member of Chromobacterium as is strikingly shown in the taxonomic analysis given by Sneath (1957b). The name Chromobacterium iodinum was validly published by Davis (though not by Clemo and McIlwaine, 1948, as no description was given). Pseudomonas clemoi is illegitimate.

I have studied two strains of this organism, the original strain of Davis (the strain here called RE) and the strain ATCC 9897 (strain TE) which is apparently derived from Davis' strain, as there seems to be no record of a re-isolation of this organism. The results are given in detail in Appendix I and the methods used were the same as those used for Chromobacterium. Strain RE was not as fully studied but it is clearly the same organism as TE, differing only in nitrate reduction.

Description of strain TE (ATCC 9897, NCTC 9742).

Morphology. A nonmotile rod, about 0.8 x 2 μ, strongly Gram-positive in young cultures but weakly so in older cultures. It shows some pleomorphism and barred staining, but no metachromatic granules, spores or capsule, and no fat droplets. Arranged in irregular or palisade-like clumps, Not acid fast. (Strain RE had the same morphology.)

Dr. W.C. Haynes finds it Gram-negative or only weakly Gram-positive, (personal communication), so it is perhaps best termed Gram-variable.

Cultural behaviour. On nutrient agar plates at 25° the colonies are round, convex, smooth, shiny, with entire edge, grey and semi-opaque. about 0.5 mm in diameter after 24 hours. After several days, when larger, they might show iodine-like crystals of the pigment. These are dark violet needles with a metallic reflex. They were seen only occasionally on nutrient agar, more often on blood agar and peptone agar, and RE produced them more often than TE (see below). Colonies are butyrous. On blood agar there is a wide zone of hazy haemolysis. Nutrient agar slants (2 days, 25°) show semi-opaque grey growth and pigment may form later. Gelatin stab (20°, 7 days): moderate stratiform liquefaction, a thin pellicle and scanty filiform growth in the depth. Nutrient broth: slight turbidity, no ring or pellicle, slight powdery deposit. Löffler serum: no digestion in 14 days at 25°. Potato: raised moist shiny grey growth with no pigment.

Resistance. It is mildly thermoduric, as a few survivors were found in a loopful of broth culture after 15 minutes at 56°, and cultures are not sterile after 30 minutes at 56°. It has the usual resistance to phenol, and is sensitive to penicillin.

Metabolism and nutrition. Strictly aerobic, grows from 10° to 37°, optimum c. 35°, grows from pH 6 to pH 10. Grows profusely on 6.5% NaCl agar. Produces no HCN, and grows very slowly in Koser's citrate, and gives late alkalinity on Simmon's citrate agar.

Carbohydrates. No acid or gas from any carbohydrate either in peptone water or Hugh and Leifson medium.

Biochemical. Litmus milk: slight alkalinity and a small clot. Indole -, NH_3 -, MR -, VP -, H_2S -, MB reduction weak, nitrate reduction of strain TE negative, but strain RE was positive, destruction of nitrite negative with both TE and RE, catalase +, urease -, phosphatase +, aesculin hydrolysis -, casein hydrolysis -, gelatin hydrolysis strong, starch hydrolysis -, haemolysis +, egg-yolk reaction -, malonate -, phenylpyruvate -, gluconate test -, arylsulfatase -, chitin digestion -.

Pigment. Strain RE gave a greater yield of iodinin than TE. The pigment is not readily formed on some media. Thus 1% peptone agar containing 0.5% of NaCl gave only a few crystals, although several brands of peptone were tried. However this was sufficient to work up, and the bacteria and crystals were washed off with water, the dark crystals separated by decantation and then recrystallized three times from chloroform. They were small dark-purple needles, M.Pt. 235° (decomposing) and the mixed melting point with an authentic sample of iodinin kindly sent by Prof. McIlwain, was also 235° (dec.). A few milligrams were weighed on a microbalance and dissolved in chloroform. The absorption spectrum is shown in Fig. 11 and the Molecular Extinction Coefficients (Mol. Wt. 244.2) are listed in Chapter X (see also Sneath, 1956b). These differ somewhat from those found by Clemo and McIlwain (1938) and Yosioka and Kidani (1952b) which are internally inconsistent and probably contain errors in the scale of the graphs, but agree with the data of Gilman (1953, allegedly an aqueous solution though it is insoluble in water). The authentic sample gave the same curve as my own sample. A Unicam spectrophotometer was used.

A larger amount of pigment was later prepared. Davis (1939) stated that citrates of sodium or potassium (but not ammonium citrate) increased pigmentation. I found no effect. Dr. B. E. Volcani informs me that glutamic acid also increases pigmentation (personal communication). Dr. Ellen Garvie recommended two media which gave good pigment formation. They were (1) Bacteriological Peptone (Evans Medical Supplies Ltd., Liverpool) 1 g, Lab-Lemco 1 g, NaCl 0.5 g, Yeastrel (Brewers Food Products Ltd., Edinburgh) 0.3 g, glucose 0.5 g, agar 2 g, water 100 ml, pH adjusted to 7.0 and autoclaved. (2) Unhopped stout beerwort 50 ml (heated at 100° for 30 minutes and filtered), water 50 ml, pH adjusted to 6.8, agar 2 g added and autoclaved. The second gave better results. From 10 litres of this in large flat bottles, inoculated to give confluent growth and incubated at 30° for 3 days and then 20° for a further 6 days, I obtained (by the technique described above) about 350 mg of iodinin. A second pigment was also found, though probably it was not present in the culture but was a breakdown product formed during the crystallization. This was very soluble in chloroform. It was recrystallized from methanol (in which iodinin is almost insoluble) and formed orange-golden crystals and plates. These had M.Pt. 271° and gave a diacetyl derivative (pale yellow needles, M.Pt. 234°). It is presumably 1:6-dihydroxyphenazine, formed by loss of the N-oxide oxygen atoms of iodinin (see Clemo and Daglish, 1950) or it may be a precursor of iodinin.

Chromobacterium orangium. See No. 51.

No. 62. Bacillus Lacmus Schroeter, 1886, p. 158.
(Bacillus Lacmus de Toni and Trevisan in Saccardo, 1889, p. 978; Godfrin, 1934, pp. 39, 214. Bacillus lacmus Thiry, 1900, p. 101; Bergey et al., 1939, p. 95; Breed et al., 1948, p. 233. Bacterium Lacmus de Lagerheim, 1891, p. 77). Latinized noun, litmus.

This organism was discovered by Schroeter growing upon window frames which had been freshly painted with oil paint. It was a slender rod which produced a purple pigment which became red with acids and blue with alkalis, like litmus. Schroeter considered it distinct from his Bacteridium cyaneum (No. 56), evidently on account of its shape. de Lagerheim believed that he had rediscovered it, but quoted Hansgirg as saying that it was an alga. It is quite unrecognizable, but the name is validly published.

No. 63. Bacillus lilacinus Macé, 1913, Vol. 2, p. 416.
(Bacillus lilacinus Waeldele, 1938, p. 41; Breed et al., 1948, p. 233). Latinized adj. lilac-coloured, pale violet.

This organism was a nonmotile bacillus which liquefied gelatin slowly, producing a tough lilac pellicle. Serum was very slowly liquefied. The colonies on agar were mauve to rose and those on gelatin were violet-lilac. The growth on potato was scanty and was described as amaranth or wine-red. Macé considered it different from Bacillus violaceus, but as he did not give the solubility of the pigment it is not certain whether it was a strain of Chromobacterium. It is therefore incertae sedis.

Brisou (1958) lists what is presumably this organism (under the name of Chromobacterium lilacinus [sic] as being agarlytic, but refers to no literature: this combination is a nomen nudum as neither description nor reference to a description is given. It was isolated from water. Macé's name is validly published, but not the name used by Brisou.

No. 64. Chromobacterium maris-mortui Elazari-Volcani, 1940, pp. VII, 76.
(Chromobacterium maris-mortui Breed et al., 1948, p. 234. Chromobacterium marismortui Breed, Eltinge and Tobie in Breed et al., 1957, p. 295. Noun, gen., Latin, of the Dead Sea.

This organism was isolated from the Dead Sea and is halotolerant, growing in media containing 0.5% to 30% sodium chloride. It is a Gram-negative bacillus about 0.5 μ in breadth and 1.5 to 13 μ in length. It is motile by peritrichous flagella. It liquefies gelatin very slowly and produces on nutrient agar containing 12% of salt a blue pigment which diffuses very little. The colonies also contain a brownish pigment. The bacillus is aerobic, produces acid without gas from several carbohydrates, reduces nitrates to nitrites, and is indole-negative. The optimum temperature is 30° and the optimum salt concentration is 12%. The pigment has not been studied extensively (Elazari-Volcani, personal communication). The taxonomic position of this organism is still uncertain: there is insufficient evidence for including it in Chromobacterium.

No. 64a. Micrococcus polychromus Makarova, 1949 cited by Krassilnikov, 1949, p. 270.

I have not been able to consult the original description. It was a large coccus producing (but only on carbohydrate-containing media) a blue or blue-green diffusing pigment, which was, however, said to be insoluble in water and other solvents. Gelatin was liquefied. Alkalis destroyed the blue-green colour. It is incertae sedis.

No. 65. Micrococcus pseudo cyaneus Schroeter, 1886, p. 145. (Micrococcus pseudo cyaneus Godfrin, 1934, p. 31. Micrococcus pseudocyaneus Macé, 1889, p. 357; 1897, p. 438, 1901, p. 438. Micrococcus pseudocyaneus Thiry, 1900, p. 98; Migula, 1900, p. 188; Breed et al., 1948, p. 272. Micrococcus cyaneus Cohn, 1872, p. 157 in part). Greek, false, and blue, Latinized: false blue micrococcus.

This organism was one of Cohn's strains which he called Micrococcus cyaneus, and which differed from Schroeter's strain of Micrococcus cyaneus (Bacteridium cyaneum Schroeter, No. 56), in that the pigment was green in alkaline solution instead of blue. It was a coccus. Cohn gave very few details of it, and Schroeter (1886) does not add any more. This organism is unrecognizable. The name appears to be validly published, by Schroeter, as it is not a trinomial, though it is not certain if he ranked it as a species or a variety (he called it var. Micrococcus pseudo cyaneus).

No. 66. Micrococcus sublilacinus Migula, 1900, p. 205. (Micrococcus sublilacinus Matzuschita, 1902, p. 222. Coccen No. 26, Lembke, 1896, p. 317; Breed et al., 1948, p. 277). Latinized, somewhat lilac-coloured; less than pale purple.

This organism was a Gram-positive diplococcus isolated from faeces, which produced a lilac pigment on potato but not on agar or gelatin. It was indole-negative, and produced acid without gas from glucose. Lembke's Coccen No. 25 was similar, but gave a lilac growth on agar, and this is probably what Migula (1900, p. 204) called Micrococcus aqueus. Neither are now recognizable. The name is validly published.

No. 67. Merismopoedia violacea Kützing, 1849, p. 472. (Agmenellum violaceum De Brébisson in lit in Kützing, 1849, p. 472. Lampropedia violacea de Toni and Trevisan in Saccardo, 1889, p. 1048; Breed et al., 1948, p. 844. Pediococcus violaceus Trevisan, 1889, p. 28; Breed et al., 1948, p. 250). Latin adj., violet-coloured.

This was a large coccus arranged in tetrads, and single cells were noticeably violet. It now seems unrecognizable. Kützing's name was validly published.

No. 67a. Pseudobacterium violaceum Krassilnikov, 1949, p. 223.

From the brief description this seems similar to No. 63. It is attributed to Kriss, but I have not been able to consult the original. It is incertae sedis. From soil. Gelatin was not liquefied.

No. 68. Bacillus Violaceus Flavus McFarlane, 1895, p. 939. (Bacillus Violaceus Flavus McFarlane in Norris and Oliver, 1897, Vol. 2, p. 525). Latin adjs., violet-coloured and yellow.

This was a nonsporing motile coccobacillus, often arranged in short chains. It was Gram-negative. It gave a stratiform liquefaction in gelatin stab culture. On agar and on gelatin it produced a diffusing violet pigment, while the colonies themselves were yellow. In broth a violet pellicle formed. On potato the growth was pink. It was isolated from the eye but was not considered to be a pathogen. Its identity is uncertain. The name is an invalid trinomial.

No. 69. Bacillus violaceus sacchari Dyar, 1895, p. 369. Latin adj., violet-coloured and Latinized noun, gen., of sugar: violet bacillus of sugar.

This was a small motile bacillus, liquefying gelatin, coagulating milk and producing a fluorescent green pigment. In media containing carbohydrates it produced a violet-black pigment. Dyar identified it with Agar's bacillus (No. 23) but it differed in several respects. It is unrecognizable. The name is an illegitimate trinomial.

No. 69a. Arthrobacter sp., Starr, 1958, p. 332.

This unnamed and undescribed species of Arthrobacter is reported by Starr to produce indigoidine, identical with the pigment of the "indigo bacteria" (Nos. 34-39) and Corynebacterium insidiosum (No. 43) and Erwinia chrysanthemi (No. 51b).

No. 70. Bacillus of Günther and Spitta, 1899, p. 108. (Bacillus de Günther and Spitta Thiry, 1900, p. 105; Godfrin, 1934, pp. 64, 223).

This bacillus is also unrecognizable. It was isolated from water and was a motile rod with a single polar flagellum. It formed deep Prussian blue colonies which developed a metallic lustre. At 37° it grew but produced no pigment. It died before the solubilities of the pigment could be determined. It may have been an "indigo bacterium."

No. 71. Bacterium h, Rosenberg, 1886, p. 458. (Bacterium h Rosenberg Thiry, 1900, p. 106. Bacterium H Rosenberg Godfrin, 1934, pp. 65, 224).

This organism is unrecognizable. It was a motile rod, said to form spores, which liquefied gelatin and produced a blue-violet growth on gelatin and a blue growth on agar and on potato. The pigment was soluble in water. The bacillus was isolated from water.

No. 72. Blauer coccus Maschek, 1887, p. 67. (? Mikrococcus cyaneus Adametz, 1888, p. 35; Micrococcus cyaneus Migula, 1900, p. 187, in part.)

This was a nonmotile coccus, producing blue colonies on agar and gelatin. The gelatin was not liquefied. Cultures had a smell like starch paste. It fermented glucose. It is unrecognizable.

No. 73. Pigmentbildender Diplococcus Klamann, 1887, column 1347. (Diplokokkus fluorescens foetidus Eisenberg, 1891, p. 10. Diplococcus fluorescens foetidus Sternberg, 1893, p. 596. Streptococcus foetidus Migula, 1900, p. 38; Matzuschita, 1902, p. 218; Breed et al., 1948, p. 340. Streptococcus fluorescens Chester, 1901, p. 70.)

Klamann described a motile, weakly Gram-positive diplococcus from the nose in ozaena. It produced a greenish pigment becoming violet in old cultures: with acids it became red. It is unrecognizable. Migula's name is validly published.

No. 74. Stamme BL, Scholl, 1889, p. 813.

This was a violet chromogenic bacterium sent to him as one of a collection of organisms isolated from blue milk, and very briefly described. It is unrecognizable. The strain was one of Hueppe's.

No. 75. Violet, Lecoq de Boisbaudran, 1882, p. 562.

This violet chromogen had been observed by Lecoq de Boisbaudran for fifteen years, growing on starch paste, and was possibly the first violet chromogen discovered. However it is not described or named, and the only details given are that the pigment was insoluble in water, soluble in alcohol and had absorption bands and colour reactions somewhat similar to that of violacein. It is uncertain if it was a bacterium, and it is therefore incertae sedis.

No. 76. Violet Bacillus, Hartley, 1913, p. 63.

This organism was isolated from water. It was a bacillus which varied in its liquefaction of gelatin, and did not form spores. Hartley reported it to be similar to Bacillus violaceus Macé. It seems to have been very similar to Chromobacterium, apparently even producing hydrogen cyanide, judging by its action on picric acid paper, but the description of the pigment is not in good agreement with the properties of violacein, and for this reason the organism is of doubtful systematic position. The violet pigment was said to show maximal absorption in alcoholic solution at about 620 mμ (for violacein it is at 580 mμ) and to be soluble in chloroform.

No. 77. Violetter Coccus, Maschek, 1887, p. 68.
(Micrococcus violaceus Migula, 1900, p. 186, in part;
Calderini, 1925, p. 773 in part).

This organism was found three times in water. It was a nonmotile coccus, often in chains, which did not liquefy gelatin. It produced a violet pigment, but this is not further described, and the organism is unrecognizable.

IV. BACTERIA WHICH APPEAR TO BE STRAINS OF CHROMOBACTERIUM.

IV (a). Bacteria recognizable as mesophilic strains of Chromobacterium (Nos. 78-91).

IV (b). Bacteria recognizable as psychrophilic strains of Chromobacterium (Nos. 92-113).

IV (c). Chromobacterium, but of uncertain species (Nos. 114-142).

In deciding whether an organism belongs to the genus a certain amount of latitude has been allowed in interpreting the old descriptions. For instance, the opinions of authors have been given more weight if the authors were familiar with these organisms, although they may not have given very full details of the organism or of its pigment. The descriptions have been kept fairly brief, and it is to be understood that the bacteria possessed the salient features of the genus (e.g. they were Gram-

negative aerobic rods producing what was apparently violacein) unless otherwise stated. The observed reactions of the pigment are not listed if they were in agreement with those given by violacein.

In deciding whether a bacterium was a mesophil or a psychrophil all the recorded details have been considered. Thus a strain has been classified as a mesophil if it possessed such features as rapid power of liquefying gelatin and growth anaerobically (which are typical of mesophils) even though its growth at 37° was not recorded or, occasionally, even if it did not grow at 37° (a feature dependent on the conditions of testing), provided the description does not clash with what is known of mesophils. For psychrophils the main details of use are the action on gelatin, oxygen requirement, growth at low temperatures, action on glucose, presence of gelatinous growth and behaviour in milk. Many strains have been classified as of uncertain species—although one can guess the more probable species—in order to avoid confusion and argument upon nomenclature.

IV (a). Bacteria recognizable as mesophilic strains of Chromobacterium.

No. 78. Bacterium janthinum Lehmann and Neumann, 1896, Vol. 1 Taf. 27, Vol. 2, p. 264.
(Bacterium violaceum Lehmann and Neumann, 1899, Vol. 1, Taf. 23, Vol. 2, p. 262; 1920, Vol. 1, Taf. 31, Vol. 2, p. 403; 1927; Vol. 1, Taf. 23, Vol. 2, p. 463; Cunningham and Raghavachari, 1924, p. 1285. Chromobacterium violaceum Breed et al., 1948, p. 231 in part). From Greek, violet-coloured, Latinized adj.

This organism was originally cited as being Zopf's bacillus (No. 115). The description is concise but adequate. The rods were 0.5-0.8 μ by 1.5-5 μ, motile by flagella which were sometimes polar sometimes peritrichous. It was said to be Gram-positive (but so was Proteus!). They were dubious about the presence of spores. The colonies on agar and gelatin were violet. It formed acid without gas from glucose. It liquefied gelatin rapidly, grew in the depth of gelatin stab cultures, and clotted and peptonized milk. The optimum temperature was the "usual temperature." The pigment was clearly violacein.

Although the growth at 37° is not recorded, this seems to have been a mesophilic organism from its behaviour in gelatin stab and in milk. The name is validly published.

No. 79. Bacterium janthinum Cruess-Callaghan and Gorman, 1935, pp. 216, 218, Pl. 6 figs. 1, 2.
(Chromobacterium ianthinum Bergey et al., 1939, p. 93; Breed et al., 1948, p. 232. Chromobacterium janthinum Breed, Eltinge and Tobie in Breed et al., 1957, pp. 292, 295.) From Greek, violet-coloured, Latinized.

This species consisted of four strains. They were Gram-negative rods measuring 0.2 to 0.3 μ by 1 to 3 μ, usually occurring singly. They were motile by means of five to seven peritrichous flagella. Gelatin

was rapidly liquefied, and milk peptonized. They gave deep violet growths on agar and potato. They were indole-negative, and reduced nitrates slightly or not at all. Glucose but not maltose, sucrose or lactose gave acid witouut gas. They grew well at 37° and did not grow at 2 to 4°. The pigment was clearly violacein. These strains were mesophilic strains of Chromobacterium. The name used by Cruess-Callaghan and Gorman is validly published. They attributed it to Zopf, but without giving reasons for this (see No. 115).

No. 80. Chromobacterium laurentium Leifson, 1956a, p. 60; 1956b, p. 399, figs. 11, 12.
From town of Lawrence, Massachusetts, Latinized.

This species consists of mesophilic strains of Chromobacterium which do not ferment carbohydrates anaerobically but only aerobically (Leifson's Group 2). It differs from Chromobacterium manilae (see No. 81) also in that the range of carbohydrates attacked is restricted, and even aerobically it only produces acid from glucose and fructose. The wavelength of the polar flagella is somewhat shorter than that found in C. manilae. Leifson found the mean wavelength and amplitude of the polar flagella to be 2.07 μ and 0.52 μ respectively, and of the lateral flagella to be 1.35 μ and 0.44 μ respectively.

These strains are ones which I consider to belong to the same species as C. manilae and which I have called "atypical mesophils" (Sneath, 1956b). The name is validly published in Leifson, 1956b, but in Leifson, 1956a, it was a 'suggested name' and this may not constitute valid publication. It is founded on Jordan's Bacillus violaceus Laurentius (No. 87), as nomenclatural type and is cited as Chromobacterium laurentium (Migula, 1900) Leifson (1956). Leifson admits that one cannot be certain whether Jordan's organism belonged to this species, and I feel that it might have been wiser to use a new epithet. If admitted to specific rank the epithet should then be purpureum, used by Eltinge (1957).

No. 81. Chromobacterium manilae Leifson, 1956a, p. 60; 1956b, p. 399, figs. 1-8.
From city of Manila, Philippine Is., Latinized.

This species was established for mesophilic strains of Chromobacterium which ferment carbohydrates anaerobically ("typical mesophils" of Sneath, 1956b, Leifson's Group 1). Leifson found that all strains were Gram-negative rods, possessing as a rule both polar and peritrichous (lateral) flagella, and they were rather smaller then the psychrophils (No. 109). They were indole-negative and urease-negative. Those mesophils which only produced acid from glucose aerobically were placed in another species (No. 80). Strains of C. manilae were able to produce acid without gas from glucose, fructose, mannose and inulin, and sometimes sucrose, but not from lactose, arabinose, xylose, mannitol, sorbitol, dulcitol, or salicin. They liquefied gelatin rapidly, and generally reduced nitrate to nitrite. They were divided into two groups, 1a, which did not utilize citrate promptly as sole carbon source and had polar flagella of average wavelength 2.13 μ, and 1b, which utilized

citrate promptly and had polar flagella of average wavelength 2.34 μ, but they were not given names. My findings are in close agreement with Leifson's. Leifson found, for subgroups 1a and 1b together, that the mean wavelength and amplitude of the polar flagella was 2.21 μ and 0.55 μ respectively and of the lateral flagella 1.31 μ and 0.46 μ respectively. The name is validly published in 1956b (possibly not in 1956a, see No. 80), and is based on Woolley's bacillus (No. 88) as nomenclatural type. It was cited as C. manilae comb. nov.; it should be cited either as C. manilae (Krassilnikov, 1949, p. 389) Leifson or as C. manilae (Schattenberg and Harris, 1942, p. 509) Leifson (see No. 88; the rule is of uncertain application on raising a variety to a full species). The neotype strain of C. violaceum (strain MK, see Judicial Commission, 1958a; 1958b) belongs to C. manilae which thus becomes an illegitimate synonym of C. violaceum.

No. 82. Bacillus violaceous [sic] Minett, 1913, p. 44.
Latin adj., violet-coloured.

This organism was isolated from milk and from water in British Guiana. It was a short Gram-negative motile nonsporing rod, often slightly curved, which produced deep violet colonies on agar. It liquefied gelatin rapidly and digested coagulated serum. It was said to be a strict aerobe. It produced acid from glucose, fructose and galactose but not from the other usual carbohydrates. It reduced nitrate to nitrite and was H_2S negative. The pigment was clearly violacein. On injection into laboratory animals it produced fatal septicaemia. These strains were clearly mesophilic strains of Chromobacterium. Though misspelt the name is validly published.

No. 83. Bacillus violaceus Eisenberg, 1888, p. 8.
(Bacillus violaceus Eisenberg, 1891, p. 91; de Lagerheim, 1891, p. 77 in part. Violetter bacillus Eisenberg, 1886, Tab. 2, No. 4).
Latin adj., violet-coloured.

This organism was from water. It was a slender rod, sometimes forming threads, motile, rapidly liquefying gelatin and liquefying blood serum. It grew on potato giving a dark violet growth. It grew in the depth of gel tin stab cultures, and was presumably facultatively anaerobic, although Eisenberg said it behaved like Bacillus prodigiosus in the absence of air, and the latter was said not to grow under a coverglass (Eisenberg, 1888, p. 3). However, Bacillus prodigiosus is a facultative anaerobe, so one need not conclude that Bacillus violaceus was a strict aerobe. It was said to form spores but this was probably an error. This organism is probably the first recognizable mesophilic strain of Chromobacterium. The powerful proteolytic action suggests this and the statement that it "does not thrive at high temperatures" does not rule this out. The name is validly published.

No. 84. Bacterium violaceum Cunningham and Raghavachari, 1924, p. 1285. Latin adj., violet-coloured.

Cunningham and Raghavachari isolated a number of strains of a violet

chromogen from water in India from several widely separated places. They identified them as Bacterium violaceum Lehmann and Neumann (No. 78). The organism was a rod measuring 0.3 to 0.5 by 1.9 to 3.9 μ, motile by peritrichous flagella, nonsporing, showing bipolar staining, Gram-negative. It produced on agar colonies which were at first yellowish and in a few days became deep violet. Gelatin was quickly liquefied. Growth was good at 37° and at 42°. On potato a deep violet growth occurred. The organism was MR-negative, VP-negative, indole-negative. Acid without gas was produced from glucose, and sometimes maltose, sucrose and dextrin. The pigment was clearly violacein. These strains were clearly mesophilic strains of Chromobacterium. The name was validly published.

No. 85. Chromobacter violaceum Morris, 1954, p. 109.
Latin adj., violet-coloured.

Morris studied a large number of strains of Chromobacterium isolated from soil and water in Trinidad. She observed that they usually grew at 37°, produced acid from glucose and often sucrose, but less often from maltose or lactose, and commonly coagulated and peptonized milk. She considered that there was no important difference between Chromobacterium violaceum and Chromobacterium ianthinum as described by Breed et al., (1948, pp. 231, 232) and suggested that they formed a single species. These were clearly mesophilic strains of Chromobacterium, and I have studied two of them (strains TV and TA). It is uncertain if the name is validly published; Chromobacter is presumably a mistake for Chromobacterium.

No. 86, Chromobacterium violaceum Eltinge, 1957, p. 43 and vars. violaceum, anitritum and purpureum Eltinge, 1957, p. 43. (Chromobacterium Groups II and III, Eltinge, 1956, pp. 142, 143. Chromobacterium janthinum Eltinge, 1957, p. 42 and vars. janthinum, anitritum and purpureum Eltinge, 1957, p. 42. Not Chromobacterium violaceum Eltinge, 1957, p. 41 (see No. 110.))
Latin adj., violet-coloured, and Latinized adj., not nitrite, and Latin adj., purple.

Eltinge studied 82 strains of the genus and on the basis of nitrate reduction divided them into three groups. This species includes the mesophilic strains (see p. 252). They "do not reduce nitrate completely beyond nitrite." They oxidize glucose and frequently fructose and sometimes sucrose. They may ferment (i.e. anaerobically) glucose, sucrose, fructose, and arabinose. They grow at 37°, some at 42°, but not at 4°. They liquefy gelatin rapidly. The variety violaceum includes the strains which ferment glucose and reduce nitrate to nitrite; var. anitritum those which ferment glucose and do not reduce nitrate; var. purpureum those which do not ferment glucose but reduce nitrate. The names are validly published, but are confusing owing to the alternative names proposed (to cover the event of a mesophil or a psychrophil being established as type species). The epithet 'purpureum' is a later synonym of 'laurentium' since this variety corresponds to No. 80.

No. 87. Bacillus violaceus Laurentius Jordan, 1890, p. 838, Pl. IV, figs. 10a, 10b, 10c.
(Bacillus violaceus Laurentius Roux, 1892, p. 308; Lustig, 1893, p. 103; Sternberg, 1893, p. 631; Voges, 1893, p. 302; Kruse in Flügge, 1896, Vol. 2, p. 312; Matzuschita, 1902, p. 136; Calderini, 1925, p. 767; Godfrin, 1934, pp. 90, 231. Bacillus violaceus laurentius Dyar, 1895, p. 372; Breed et al., 1948, p. 233. Bacillus Violaceus Laurentius Horrocks, 1901, p. 68; Waeldele, 1938, p. 37. Bacillus violaceus Chester, 1901, p. 262. Bacterium violaceus [sic] Laurentius [sic] Chester, 1897, p. 117. Bacterium Laurenti Enderlein, 1925, p. 281. Pseudomonas Laurentia Migula, 1900, p. 944; 1901, p. 384. Pseudomonas laurentia Krassilnikov, 1949, p. 387. Pseudomonas janthina Chester, 1901, p. 317 in part. Chromobacterium violaceum laurentium Ford, 1927, p. 470. Chromobacterium laurentium Leifson, 1956a, p. 60; 1956b, p. 399). Latin adj., violet-coloured, and town of Lawrence, Massachusetts, Latinized.

This strain was isolated by Jordan from a sewage effluent. It was a moderate-sized, motile, nonsporing rod, often in pairs, sometimes forming short chains. The growth on agar and potato was deep violet. Nitrates were said to be reduced to nitrites rather slowly. It liquefied gelatin rapidly, and the violet colonies were surrounded by a narrow fluid zone. Milk became acid and was coagulated. It apparently grew at 37° since Jordan said that it grew better at 21° than at 37°, and it was apparently able to grow anaerobically, since Jordan said that it grew well under a plate of mica. Leifson (1956b) found it difficult to get conclusive results with his strains when growing them under glass plates, and my own experience has also been inconclusive, though in general the strictly aerobic psychrophils grew more poorly than mesophils.

This bacterium was clearly a mesophilic strain of Chromobacterium. Jordan's name is an invalid trinomial. The first valid name is Migula's.

No. 88. Bacillus violaceus Manilae Woolley, 1904, p. 1; 1905, p. 89. (Bacillus violaceus Manillae [sic] Waeldele, 1938, p. 35. Bacillus violaceous [sic] Minett, 1913, p. 44. Chromobacterium violaceum Manilae Ford, 1927, p. 471. Chromobacterium violaceum manilae, Breed et al. 1948, p. 234. Chromobacterium violaceum var. manilae Schattenberg and Harris, 1942, p. 509. Chromobacterium manilae Leifson, 1956a, p. 60; 1956b, p. 399. Pseudomonas manilae Krassilnikov, 1949, p. 389). Latin adj., violet-coloured, and city of Manila, Philippine Is., Latinized.

This organism was isolated by Woolley from water buffaloes which died of a generalized infection. Woolley's description is a good one, and may be summarized as follows. It was a rod measuring 0.5 μ x 1-1.5 μ, Gram-negative, nonsporing though clear spaces may be seen in the rods, motile by one or rarely two polar flagella. It produced violet colonies on agar at 37°. Gelatin was liquefied and it grew on potato giving a deep violet growth. In broth turbidity and a violet pellicle appeared. Milk was peptonized. It was almost an obligate aerobe. The pigment was

soluble in alcohol but scarcely soluble in water, ether or chloroform. It was virulent for laboratory animals on injection. The optimum temperature for growth and pigmentation was 37°. The organism was certainly a mesophilic strain of Chromobacterium. Woolley's name is a trinomial and is illegitimate, as is also Ford's name. The name used by Schattenberg and Harris and those used by Leifson and Krassilnikov are validly published.

No. 89. Bacillus violarius acetonicus Bréaudat, 1906, p. 879. (Bacillus violarius acetonicus Godfrin, 1934, pp. 91, 232. Bacillus violaceus [sic] acétonicus [sic] Guillerin, 1929, p. 38. Bacillus violaceus [sic] acetonicus Lehmann and Neumann, 1920, Vol. 2, p. 476; Broudin, 1922, p. 197; Calderini, 1925, p. 772. Bacillus Violarius acetonicus Waeldele, 1938, p. 38. Aerobacillus violarius Donker, 1926, p. 142. Variety of Bacillus polymyxa Bergey et al., 1939, p. 703; Breed et al., 1948, p. 720). Etym. dubious; ? From Latin violarium a bed of violets, and acetone, Latinized.

Bréaudat described a Gram-positive rod isolated from water in Saigon. It was motile, showed bipolar staining, liquefied gelatin, reduced nitrate to nitrite and in litmus milk gave acid, clot and peptonization. It produced a deep violet pigment on agar. It grew well at 37°. It formed small amounts of acetone during growth in peptone water. Bréaudat reported that it formed spores about the sixth day of incubation, but did not test their heat resistance. The description is in good agreement with that of a mesophilic strain of Chromobacterium, and evidently this is what it was. The spores were presumably artifacts or vacuoles. Bréaudat's name is an illegitimate trinomial. Donker's name is validly published. It is possible that 'violarius' is a misprint for 'violaceus', as Broudin and Guillerin, who worked at Saigon, used the latter form.

No. 90. Bacille bleu van der Sleen, 1894, No. 26, Pl. VII, fig. 26.

This organism was a rod, sometimes forming chains. Motility was doubtful. It grew at 37°, liquefied gelatin rapidly, and grew in the depth of gelatin stab cultures. It gave a deep violet growth on agar and on potato. The pigment was evidently violet, and was described as 'like a violet', and it evidently did not diffuse in agar. This strain was probably a mesophilic strain of Chromobacterium.

No. 91. Bacille violet pathogène Gauducheau, 1907, p. 278. (Bacille violet pathogène de Gauducheau Godfrin, 1934, pp. 104, 238; Waeldele, 1938, p. 46).

This organism was isolated from well water in Indo-China, and was virulent for animals on injection. It was a motile Gram-negative rod, often slightly curved, producing deep violet growth on agar and potato. Gelatin was rapidly liquefied, and milk was clotted and peptonized. It grew at 37°. The pigment was clearly violacein. It was certainly a mesophilic strain of Chromobacterium. Gauducheau was the first to notice the curious smell of cultures of mesophils, which he said smelt

of bitter almonds, due to the production of large amounts of hydrogen cyanide (see Chapter XIII).

No. 91a. Other mesophilic strains.

The following organisms described in more recent years are so clearly mesophils that individual descriptions of them are not given, especially as the papers do not claim to be taxonomic studies. Strains which I have studied are listed in brackets.

Chromobacterium violaceum Martin, 1931, p. 68; Floch and de Lajudie 1943, p. 2; Sneath et al., 1953, p. 276 (strains BH, BN and many others); Audebaud et al., 1954, p. 416; Sippel et al., 1954, p. 468 and Sippel, 1955, p. 1 (strains SL, RV, PT); Darrasse et al., 1955, p. 704 (strain DK); Joubert and Nguyem-Van-Liem, 1957, p. 341. Chromobacterium violaceum var. manilae Schattenberg and Harris 1942, p. 509. Bacillus violaceus Ramchandani, 1930, p. 975; Black and Shahan, 1938, p. 1270 (strain SH), Soule, 1939, p. 592; Dodd, 1941, p. 19 (strain SH). Bacillus violaceous [sic] Lesslar, 1927, p. 28.

IV (b). Bacteria recognizable as psychrophilic strains of Chromobacterium.

No. 92. Chromobacterium bamptonii Bergey et al., 1923, p. 119. Named for J. H. Bampton.
(Chromobacterium bamptonii Bergey et al., 1926, p. 124; 1930, p. 159; 1934, p. 171; 1939, p. 94; Breed et al., 1948, p. 234. Bacillus membranaceus amethystinus Stamm II Bampton, 1913, p. 137. Bacillus membranaceus amethystimus [sic] II Godfrin, 1934, pp. 103, 237. Bacillus Membranaceus Amethystinus II Waeldele, 1938, p. 44. Chromobacterium membranaceum amethystinum II Ford, 1927, p. 473. Chromobacterium amethystinum Krassilnikov, 1949, p. 501.)

This species based on Bampton's Stamm II was very similar to Chromobacterium membranaceum (No. 99) and appears to have been a psychrophilic strain. C. bamptonii is a validly published name.

No. 93. Chromobacterium cohaerens Grimes, 1930, p. 383. (Chromobacterium cohaerens Bergey et al., 1939, p. 95; Breed et al., 1948, p. 234.) Latin part., clinging together.

This species was founded on a single strain isolated from water. It was very similar to Grimes' C. hibernicum (No. 114), the main point of difference being the tough membranous growth. It was also diastase-positive. Although it liquefied gelatin within 10 days it was evidently a membranous psychrophil. The name is validly published.

No. 94. Bacillus janthinus Plagge and Proskauer, 1887, p. 464. (Bacillus janthinus Lustig, 1890, p. 89 in part. Bacillus ianthinus Lustig, 1893, p. 76 in part. Bacillus lividus Eisenberg, 1891, p. 81; Roux, 1892, p. 303; Germano, 1892, p. 517; Voges, 1893, p. 303;

Sternberg, 1893, p.621; Chester, 1901, p.262; Matzuschita, 1902, p.140; Calderini, 1925, p.769; Godfrin, 1934, pp.97, 235; Waeldele, 1938, p.42; Krassilnikov, 1949, p.634. ? Bacillus violaceus Berolinensis Kruse in Flügge, 1896, Vol.2, p.311 in part. ? Bacillus Violaceus Berolinensis Horrocks, 1901, p.68 in part. ? Bacillus Berolinensis Chester, 1901, p.305, not Bacillus Berolinensis Migula, 1900, p.856, not Bacillus berolinensis Macé, 1913, Vol.2, p.411 (No.36). Bacterium lividum Migula, 1900, p.399 in part; Bacterium lividus [sic] Chester, 1897, p.117. Bacterium jochromum de Lagerheim, 1891, p.77 in part. Pseudomonas violacea Pribram, 1933, p.49 in part. Chromobacterium lividum Holland, 1920, pp.219, 222; Bergey et al., 1923, p.119; 1926, p.124; 1930, p.158; 1934, p.170; 1939, p.94; Breed et al., 1948, p.234; Eltinge, 1957, p.42). From Greek, violet-coloured, Latinized.

This strain was isolated by Plagge and Proskauer from water at Berlin, and they considered it to be "Bacillus janthinus Zopf" (see No. 115). It produced an inky blue growth on agar and on potato, liquefied gelatin very slowly, and grew poorly in the depths of the gelatin stab. Although the description is brief, this was evidently a strain of Chromobacterium. and Plagge and Proskauer made it plain in their discussion that they were referring to violet organisms although they did not explicitly state whether the pigment was blue or violet. Eisenberg (1891) in redescribing the bacillus, mentioned that the pigment was blue-black and violet-blue. Plagge and Proskauer also described, without naming it, another violet chromogen which liquefied gelatin rapidly, which they refer to as being well known. This has confused the nomenclature, as most subsequent authors do not clearly say which they are referring to. It is clear that Plagge and Proskauer had noticed the difference between strains which liquefy gelatin quickly and those which liquefy it slowly, and unlike other authors, had studied both, and this organism is therefore the earliest which can be recognized with any degree of certainty as a psychrophilic strain of Chromobacterium. Bergey et al. (1923, p.119) stated that its optimum temperature was 35°, but there is no evidence that this was so.

The name used by Plagge and Proskauer was validly published but it is a later homonym of Bacillus janthinus Flügge, 1886 (No.117) which is unrecognizable as to species. Eisenberg's name Bacillus lividus appears to be the earliest valid epithet for this organism and for a recognizable psychrophil (see Sneath, 1956a). I proposed in that paper that strain HB (NCTC 9796, ATCC 12473) be the neotype strain (see p. 339).

The specific epithet 'jochromum' used by de Lagerheim appears to have been published later in 1891 than Eisenberg's name, since there is a notice of the third edition of Eisenberg's book in the Zentralblatt für Bakteriologie Vol.9, No.14 (issued on 16 April, 1891) p.488, and a review of the book in No. 20 (issued on 23 May, 1891) pp.677-678, while de Lagerheim's article is dated 9th July, 1891, and must therefore have been effectively published on or after that date. In addition, de Lagerheim's species Bacterium jochromum is based on both the organism of Flügge and of Plagge and Proskauer, and it is uncertain which is the nomenclatural type or whether the name is invalid on this account.

No. 95. Bacillus janthinus Macé, 1889, p. 528.
(Bacillus janthinus Macé, p. 855; 1901, p. 920; 1913, Vol. 2, p. 414. Bacille violet Macé, 1888c, p. 526). From Greek, violet-coloured, Latinized.

This organism was isolated from water. It was a rod measuring 0.6 x 1.8 to 2 μ, arranged singly or in pairs, motile, and liquefying gelatin only after several weeks. It gave a brownish growth on potato. It grew slowly and pigmented slowly. Macé stated that the violet pigment was identical with that of Bacillus violaceus, and noted that it was very similar to Bacillus lividus (No. 94) and Bacillus membranaceus amethystinus (No. 100) (Macé, 1901, p. 920). It appears to have been a psychrophilic strain of Chromobacterium. The name is validly published.

No. 96. Bacillus janthinus Zimmermann, 1890, pp. 84(36), 140(92); 1893, Taf. II, fig. 13.
(Bacillus janthinus Eisenberg, 1891, p. 420 in part; Voges, 1893, p. 303 in part; Godfrin, 1934, pp. 95, 234 in part. Bacillus Janthinus Waeldele, 1938, p. 40 in part. Bacillus ianthinus Lustig, 1893, p. 76 in part. Pseudomonas ianthina Migula, 1900, p. 941 in part; Krassilnikov, 1949, p. 387 in part). From Greek, violet-coloured, Latinized.

This was isolated from water at Chemnitz. The bacillus measured 0.65 x 1.5-3.5 μ, and the rods were often slightly curved. It was motile, liquefied gelatin slowly, grew poorly in broth giving slight turbidity and a pellicle with a ring at the junction with the side of the tube. It was strictly aerobic, grew slowly, and room temperature was the optimum. On solid media it produced a violet pigment after several days growth. The Gram-reaction is not recorded but the figure (Zimmermann, 1893, Taf. II, fig. 13) shows barred and bipolar staining. It was clearly a psychrophil. Zimmermann's name was validly published in 1890.

No. 97. Bacillus janthinus var butyricus Deshusses and Novel, 1939, p. 7. From Greek, violet coloured, Latinized, and Modern Latin adj., relating to butter, from Greek.

This strain was found growing on butter on which it formed violet patches. It was a motile rod with peritrichous flagella, Gram-negative, showing bipolar staining and forming no spores. It grew at 2° and at 34°, liquefied gelatin slowly and did not ferment carbohydrates, and was nonhaemolytic. The violet pigment had the same solubilities and reactions with reagents as violacein. Deshusses and Novel considered that it was a new variety because it had peritrichous flagella, was indole-negative, H_2S-negative, was strictly aerobic and did not clot milk; in fact these features are not abnormal, and the strain was a typical psychrophil. The name is validly published but the varietal name is redundant.

No. 98. Bacillus lividus Zimmermann, 1893, p. 94.
(Bacillus Lividus Horrocks, 1901, p. 68, Bacterium lividum Migula, 1900, p. 339 in part. Bacillus pseudolividus Matzuschita,

1902, p.168; Calderini, 1925, p.769). Latin adj., leaden-coloured, dark blue.

This bacterium from water was a nonmotile rod, measuring 0.8 to 1.0 by 1.5 to 4.7 μ, said to possess central oval spores. Colonies were at first yellowish and later became violet in the centre. Gelatin was slowly liquefied. Growth on potato was brownish-blue and broth was rendered turbid. It grew very slowly, was strictly aerobic, and the optimum temperature was room temperature. It seems clear that this was a psychrophilic strain. Zimmermann did not attribute the name to a previous author, so it is a later homonym of Bacillus lividus Eisenberg, 1891; Matzuschita's name is validly published. Matzuschita described it as Gram-positive, which seems unlikely.

No. 99. Chromobacterium membranaceum Bergey et al., 1923, p.119. (Chromobacterium membranaceum Bergey et al., 1926, p.125; 1930, p.159; 1934, p.171; 1939, p.94; Breed et al., 1948, p.234. Bacillus membranaceus amethystinus Stamm I Bampton, 1913, p.135; Bacillus membranaceus amethystimus [sic] I Godfrin, 1934, pp.102, 237. Bacillus Membranaceus Amethystinus I Waeldele, 1938, p.44. Chromobacterium membranaceum amethystinum I Ford, 1927, p.472). Latin adj., membrane-like.

This was a typical psychrophilic strain which grew at 4° and not at 37°, liquefied gelatin very slowly and produced a membranous (gelatinous) growth. C. membranaceum is a validly published name.

No. 100. Bacillus membranaceus amethystinus Eisenberg, 1891, p. 421.
(Bacillus membranaceus amethystinus Germano, 1892, p.516; Voges, 1893, p.302; Sternberg, 1893, p.634; Frankland and Frankland, 1894, p.474; van der Sleen, 1894, No.25, Pl.VII, fig.25; Miquel and Cambier, 1902, p.695; Godfrin, 1934, pp.99, 236; Waeldele, 1938, p.43 in part. Bacillus amethystinus Kruse in Flügge, 1896, Vol.2, p.312; Macé, 1897, p.856; Matzuschita, 1902, p.238; Calderini, 1925, p.769. Bacillus Amethystinus Horrocks, 1901, p.69. Bacterium membranaceum amethystinum Cruess-Callaghan and Gorman, 1935, p.216. Bacterium amethystinum Migula, 1900, p.491; Chester, 1901, p.179. ? Bacterium amethystinum Enderlein, 1925, p.281. Bacterium amethystinus [sic] Chester, 1897, p.117. Chromobacterium amethystium [sic] Bergey et al., 1923, p.121. Chromobacterium amethystinum Holland, 1920, pp.217, 222; Bergey et al., 1926, p.126; 1930, p.161; 1934, p.173; 1939, p.94; Breed et al., 1948, p.232; Breed, Eltinge and Tobie in Breed et al., 1957, p.294. ? Chromobacterium amethystium [sic] Topley and Wilson, 1929, Vol.1, p.402; 1936, p.497; Wilson and Miles, 1946, Vol.1, p.635; 1955, Vol.1, p.730. Not Chromobacterium amethystinum Gilman, 1953, p.48. Mycobacterium ammethystinum [sic] Krassilnikov, 1941, p.102. Mycobacterium amethystinum Krassilnikov, 1949, p.171). Latin adj., membrane-like, and Latin adj., amethyst-coloured, violet.

This was found by Jolles (reference not traced) in well water at Spalato, and was described by Eisenberg. It was a nonmotile nonsporing rod showing bipolar staining. It liquefied gelatin slowly and gave colonies which were at first cream-coloured and later deep violet with a metallic sheen. The pigment evidently did not diffuse. It grew slowly at room temperature and did not grow at 37°. On potato it grew poorly, giving a dirty yellow or greenish growth. It was strictly aerobic. The growth on agar was a thick tough membrane which could be stripped off the medium, and a similar membrane was formed on the surface of broth cultures. It was clearly a gelatinous (membrane-forming) psychrophilic Chromobacterium. Eisenberg's name is an illegitimate trinomial. That of Kruse is validly published. Chester (1897;1900) misquotes Eisenberg's description, saying that it grows at 37°.

No. 101. Bacillus membranaceus amethystinus van der Sleen, 1894, No. 25, Pl. VII, fig. 25.
Latin adj., membrane-like, and Latin adj., amethyst-coloured, violet.

This strain from water was a rather large rod, producing a violet pigment. It was nonmotile and showed slight bipolar staining. It slowly liquefied gelatin, gave a violet growth on potato and on agar, and in broth produced turbidity and a white ring at the surface. It was probably a psychrophilic strain of Chromobacterium. The name is an invalid trinomial.

No. 102. Bacillus membranaceus amethystinus Bampton, 1913, p. 135, Taf. figs. 1-5.
(Chromobacterium membranaceum amethystinum I, II, III, and IV Ford, 1927, pp. 472-474). Latin adj., membrane-like, and Latin adj., amethyst-coloured, violet.

These strains differed from Bacillus violaceus Bampton (No. 108) in very few respects. They grew on potato and produced membranous growth and were of a distinct serological group. They were said to resist heating better than B. violaceus. They were clearly psychrophilic strains of Chromobacterium. The name is an invalid trinomial. The species has been divided up by other authors (see Nos. 92, 99, 103, and 104).

No. 103. Chromobacterium membranaceum amethystinum III Ford, 1927, p. 474.
(Chromobacterium membranaceum amethystinum III Breed et al., 1948, p. 234. Bacillus membranaceus amethystinus Stamm III Bampton, 1913, p. 138, Taf. figs. 1, 2). Latin adj., membrane-like and Latin adj., amethyst-coloured, violet.

This strain was a typical psychrophil (see No. 102). The name is an illegitimate quadrinomial.

No.104. Chromobacterium membranaceum amethystinum IV Ford, 1927, p.474.
(Chromobacterium membranaceum amethystinum IV Breed et al., 1948, p. 234. Bacillus membranaceus amethystinus Stamm IV Bampton, 1913, p.138, Taf., Figs.3-5). Latin adj., membrane-like and Latin adj., amethyst-coloured, violet.

This strain was also a typical psychrophil (see No.102) and the name is an illegitimate quadrinomial.

No.105. Bacterium membranaceum amethystinum Cruess-Callaghan and Gorman, 1935, pp.216, 219, Pl.6, figs.3, 4, 6, 8.
(Chromobacterium amethystinum Bergey et al., 1939, p.94; Breed et al., 1948, p.232; Breed, Eltinge and Tobie in Breed et al., 1957, pp.292, 294 pro synon. Bacillus membranaceus amethystinus Eisenberg, No.100). Latin adj., membrane-like and Latin adj., amethyst-coloured, violet.

This species consisted of nine strains. They were Gram-negative rods, 0.3-0.5 x 1.5-3 μ, usually occurring singly, motile by a single polar flagellum (though peritrichous flagella were seen in a few strains). Growth on agar and on potato was deep violet. Gelatin was slowly liquefied, there was little peptonization of milk and the pigment was evidently violacein. They were indole-negative, reduced nitrate to nitrite, produced slight or no acid from glucose, maltose and sucrose. They grew well at 2-4° and did not grow at 37°. They were clearly psychrophilic strains of Chromobacterium. The name is an invalid trinomial and is given on p.219 as Bacterium violaceum amethystinum.

No.106. Bacillus membranaceus amethystinus mobilis Germano, 1892, p.518.
(Bacillus membranaceus amethystinus mobilis Miquel and Cambier, 1902, p.690; Bergey et al., 1939, p.95; Breed et al., 1948, p.233; Bacillus amethystinus mobilis Kruse in Flügge, 1896, Vol.2, p.313; Matzuschita, 1902, p.138; Calderini, 1925, p.770. Bacillus Amethystinus Mobilis Horrocks, 1901, p.69. Bacillus membranaceus amethystinus variété "mobilis" Godfrin, 1934, p.99. Bacillus membranaceus amethystinus Waeldele, 1938, p.43 in part. Bacillus amethystinus Chester, 1901, p.262. Bacterium amethystinus [sic] mobilis [sic] Chester, 1897, p.117. Bacterium violaceum var. amethystinum Pribram, 1919, p.7. Pseudomonas amethystina Migula, 1900, p.944; 1901, p.385; Krassilnikov, 1949, p.387. ? Bacterium amethystinum Enderlein, 1925, p.281). Latin adj., membrane-like, Latin adj., amethyst-coloured, violet and Latin adj., movable, swift.

This strain was isolated from air. Germano described it as a slender long rod, which was motile. It did not grow in the thermostat (presumably at 37°). It slowly liquefied gelatin, giving a violet membranous growth, and on broth produced a rugose pellicle. It coagulated milk. The pigment appears to have been violacein. It was very similar to

Eisenberg's Bacillus membranaceus amethystinus (No. 100), but differed in that it gave a brownish growth on potato and was motile. Lehmann and Neumann (1899, Vol. 2, p. 264) and Ward (1898) considered it a variety of No. 100. This organism was clearly a gelatinous psychrophilic strain of Chromobacterium. Germano's name is an illegitimate quadrinomial.

No. 107. Mikrococcus [sic] violaceus Adametz, 1888, p. 34. (Mikrococcus violaceus Matzuschita, 1902, p. 436. Micrococcus violaceus Sternberg, 1893, p. 601; Miquel and Cambier, 1902, p. 666; ? Violetter Coccus Maschek, 1887, p. 67). Latin adj., violet-coloured.

Adametz does not make clear what strain he is describing, though he attributes the name to Cohn (1872). The description is close to that of "Violetter coccus" of Maschek (see No. 77). It was a nonmotile coccobacillus which did not liquefy gelatin, grew poorly in the depth of the gelatin stab, and gave a slimy violet growth on gelatin plates. It grew rather slowly. The name may not be validly published, since Adametz did not always use Linnaean binomials.

No. 108. Bacterium violaceum, B. violaceus Bampton, 1913, p. 129. (Chromobacterium violaceum Ford, 1927, p. 469 in part). Latin adj., violet-coloured.

These strains were nonsporing Gram-negative rods, motile, possessing polar and peritrichous flagella. Metachromatic granules were often present. They grew at 6° but not at 37° and were facultative anaerobes. They did not grow on potato. They liquefied gelatin and sometimes also inspissated serum, and sometimes peptonized milk. They were indole-negative, usually H_2S-negative, produced acid from glucose but only doubtful acid from other carbohydrates. Though distinctly proteolytic they were probably all psychrophilic chromobacteria. The name is validly published.

No. 109. Chromobacterium violaceum Leifson, 1956a, p. 60; 1956b, p. 399, figs. 9, 10. Latin adj., violet-coloured.

Leifson in his excellent description of the genus divides it into three species. This species comprises the psychrophils (Group 3). He found them to be Gram-negative rods which possessed both polar and peritrichous flagella as a rule. They produced violacein. They were all indole-negative and urease-negative. They differed from the mesophils in that they produced acid from carbohydrates only aerobically, did not grow at 37°, and liquefied gelatin slowly or not at all. They reduced nitrates to nitrite, and utilized citrate as sole carbon source. They oxidized glucose, fructose, mannose, arabinose, xylose, mannitol, and sorbitol, and sometimes sucrose and maltose, but not inulin or dulcitol. The organisms were rather larger than mesophils. Leifson found that the mean wavelength and amplitude of the polar flagella was 2.23 μ and 0.56 μ respectively, and of the lateral flagella 1.25 μ and 0.43 μ respectively.

The name is validly published, though it is incorrectly cited as Chromobacterium violaceum (Schroeter) Bergonzini, and Leifson does not say why he considered that the nomenclatural type (whatever that is, see Nos. 123, 124) was a psychrophil.

No. 110. Chromobacterium violaceum Eltinge, 1957, p. 41 and vars violaceum Eltinge, 1957, p. 41 and mesophilum Eltinge, 1957, p. 42.
(Chromobacterium Group I Eltinge, 1956, p. 142. Chromobacterium lividum Eltinge, 1957, p. 42 and vars lividum and mesophilum, Eltinge, 1957, p. 42. Not Chromobacterium violaceum Eltinge, 1957, p. 43. Latin adj., mesophil, middle temperature loving, from Greek.

Bacteria producing a violet pigment soluble in ethanol but not water which "reduce nitrate completely beyond nitrite." They oxidize glucose and maltose and usually xylose and arabinose and may oxidize sucrose. They liquefy gelatin slowly, usually grow well at 4° and usually do not grow at 37°. The variety lividum grows at 4° but not at 37°, while the variety mesophilum grows at 37° but not at 4°. The former variety are typical psychrophils. I consider the latter variety to be aberrant psychrophils with a higher temperature optimum than usual (see Chapters I and III). The names are validly published.

No. 111. Bacillus violaceus diffusus Ajtai, 1897, p. 666, Tab. VI, 10 raj.
(Stamm Violaceus Diffusus Aitay, Bampton, 1913, p. 134).
Latin adjs., violet-coloured, and spread out.

This organism was a nonmotile Gram-negative rod which did not liquefy gelatin. It produced flat effuse violet colonies with wrinkled periphery and a crateriform centre on gelatin plates. It appears to have been a psychrophil with aberrant colonial features. Bampton studied what was evidently this strain and did not find it greatly different from his other strains. The name is an invalid trinomial.

No. 112. Chromobacterium spp. Corpe, 1953, p. 470; 1954, p. 11.

Most of Corpe's strains were psychrophils isolated from soil, but three (7461, 6357 and 533 which are presumably the strains LW, SH and RT respectively in this study) were atypical mesophils. His description is based mainly on the psychrophils. These he found to be Gram-negative rods, sometimes showing Gram-positive granules, with polar or peritrichous flagella. They utilized citrate as sole carbon source, reduced nitrate to nitrite and frequently to gaseous nitrogen, were indole-negative and H_2S-negative. They generally grew at 4° and not at 37°. They produced slow peptonization of milk. A few digested egg white and hydrolyzed starch. In a synthetic medium they produced acid from glucose and commonly from arabinose, xylose, fructose, galactose and maltose, and less often from other carbohydrates. Many strains gave a tough membranous growth.

No. 113. Violet Bacillus Ward 1898, p. 59, figs. 1 to 10.
(Bacille violet de la Tamise Miquel and Cambier, 1902, p. 696.)

Ward (1897) recorded the isolation of violet chromogens from River Thames water in winter, and in 1898 described a strain in detail. It was a rod measuring 0.5-0.8 μ by 3-7 μ, motile and nonsporing. It slowly liquefied gelatin and produced a tough membranous violet growth on agar. It grew at 5° but not at 35° and slowly peptonized milk. It did not ferment glucose and was nonpathogenic on injection into guinea pigs. The pigment was clearly violacein. This was clearly a psychrophilic strain of Chromobacterium, which Ward considered to be very similar to Bacillus membranaceus amethystinus Eisenberg (No. 100) and to Bacillus violaceus Frankland and Frankland (No. 129). Ward commented that a different type might be found in the summer, but was not explicit.

IV (b) Chromobacterium, but of uncertain species

No. 114. Chromobacterium hibernicum Grimes, 1930, p. 382.
(Chromobacterium hibernicum Bergey et al., 1939, p. 95; Breed et al., 1948, p. 234). Modern Latin adj., from Latin Hibernia, Ireland; Irish.

This was isolated from water in Ireland. This species and Chromobacterium cohaerens (No. 93) were established by Grimes because his sources of information stated, quite erroneously, that the bacteria of the genus Chromobacterium do not reduce nitrate. His strains did, and the result is two redundant names. These strains were Gram-variable rods, motile, nonsporing, producing violet colonies on agar. On potato growth was brown, later violet. Gelatin was liquefied rather slowly, and carbohydrates were not fermented. They were indole-negative, reduced nitrate and diastase negative. The description is most close to that of psychrophils but since growth was recorded as "scanty or absent" at 37° and in milk they gave slight acid, slow clotting and peptonization; I have listed it as of uncertain species. The name is validly published.

No. 115. Bacterium ianthinum Zopf, 1883, p. 68.
(Bacterium ianthinum Zopf, 1885, p. 68. Bacterium janthinum Lehmann and Neumann, 1896, Vol. 1, Tab. 27, Vol. 2, p. 264; Enderlein, 1925, p. 281; Cruess-Callaghan and Gorman, 1935, p. 216. Bacterium janthinus [sic] Chester, 1897, p. 117. Bacterium violaceum Lehmann and Neumann, 1899, Vol. 1, Tab. 23, Vol. 2, p. 262; 1920, Vol. 1, Taf. 31, Vol. 2, p. 403; 1927, Vol. 1, Taf. 23, Vol. 2, p. 463 in part. Bacillus ianthinus Crookshank, 1887, p. 303; Lustig, 1893, p. 76; Schneider, 1894, p. 228. Bacillus janthinus Flügge, 1886, p. 291; Plagge and Proskauer, 1887, p. 464; Macé, 1889, p. 528; 1897, p. 855; 1901, p. 920; 1913, Vol. 2, p. 414; Lustig, 1890, p. 89; Zimmermann, 1890, pp. 84(36), 92(140); 1893, Taf. II, fig. 13; Jordan, 1890, p. 840; Tils, 1890, p. 311; Eisenberg, 1891, p. 420 in part; Sternberg, 1893, p. 631; Wright, 1895, p. 450; Kruse in Flügge, 1896, Vol. 2, p. 312; Matzuschita, 1902, p. 138; Miquel and Cambier, 1902, p. 689; Godfrin, 1934, pp. 95, 234 in part. Bacillus Ianthinus Calderini, 1925, p. 767. Bacillus Janthinus Roux, 1892, p. 301; Horrocks,

1901, p.67, Pl.IV, fig.8; Waeldele, 1938, p.40. Bacillus violaceus in part Schroeter, 1886, p.157; de Toni and Trevisan in Saccardo, 1889, p.978. Bacillus violaceus Horrocks, 1901, p.67, Pl.IV, fig.8. Pseudomonas ianthina Migula, 1900, p.941 in part, 1901, p.380; Krassilnikov, 1949, p.387 in part. Pseudomonas janthina Chester, 1901, p.317. Pseudomonas violacea Pribram, 1933, p.49 in part. Arthrobacterium ianthinum de Lagerheim, 1891, p.77. Chromobacterium janthinum Holland, 1920, p.222; Bergey et al., 1923, p.120; 1926, p.125; 1930, p.160; Breed, Eltinge and Tobie in Breed et al., 1957, p.295; Ford, 1927, p.474; Eltinge, 1957, p.42. Chromobacterium ianthinum Bergey et al., 1934, p.172; 1939, p.93; Breed et al., 1948, p.232 in part. Not Chromobacterium ianthinum Gilman, 1953, p.48, see No. 59.) Greek, violet-coloured, Latinized.

This organism was briefly described by Zopf (1883; 1885) as follows: "11. Bacterium ianthinum Zopf. I found this bacteria on a piece of pig's bladder which I had left floating in very infected water from the Panke in Berlin. It produces spots of an intense violet colour, 1-10 millimeters in diameter. It formed long and short swarming rods, later splitting into cocci. The pigment, of a beautiful violet colour, and soluble in alcohol, was formed on the surface of the pig's bladder only in direct contact with the air, never away from air on the underside of the pig's bladder which was under the water. In its oxygen requirement for pigment formation, B. ianthinum thus resembles all other chromogenic bacteria."

[11. Bacterium ianthinum Zopf. Ich erzog diesen Spaltpilze auf Stücken von Schweinsblase, die ich in stark spaltpilzhaltiges Wasser (aus der Panke in Berlin) legte, so zwar dass sie auf der Oberflache schwammen. Es bildeten sich 1-10 Millim. im Durchmesser haltende Flecken von intensiv violetter Färbung. Sie bestanden aus längeran und kürzeren schwärmfähigen Stäbchen, die schliesslich in Coccen zerfielen. Das Pigment, ein schön violetter in Alkohol löslicher Färbstoff ensteht nur an der unbenetzten, mit der Luft in direkter Verbindung stehenden Oberfläche der Schweinsblase, nie an der von der Luft abgewandten Seite und nie an untergetauchten Schweinsblasenstücken. Bezüglich seines Sauerstoffbedürfnisses für die Pigmentbildung verhält sich A. [sic, corrected to B. in the 1885 edition] ianthinum also wie alle anderen Pigmentpilze.]

The first description is from what was evidently the first edition of Zopf's Die Spaltpilze, published as a separate from the Encyklopaedie der Naturwissenschaften, dated 1883 and reprinted in the Encyklopaedie in 1884. The third edition is dated 1885 and is a separate from the Encyklopaedie. I have not been able to trace a second edition, though Breed et al. (1948, p.232) quote it. The name was validly published in 1883.

No more information is available on Zopf's strain, and most of the descriptions are accretions based upon the strains of Flügge (1886) and many others. It was probably a strain of Chromobacterium but the species is uncertain. Zopf's name is validly published. There has been much confusion in the use of the epithet ianthinum; many authors, e.g. Macé (1888c), Jordan (1890), use the name for psychrophilic strains which liquefy gelatin slowly, while Cruess-Callaghan and Gorman (1935)

use it for typical mesophils but without any explanation of why they considered these were of the same species as Zopf's organism.

No.116. Bacillus janthinus Lustig, 1890, p.89.
(Bacillus ianthinus Lustig, 1893, p.76). Greek, violet-coloured, Latinized.

This species was based on the descriptions of organisms of Flügge, Plagge and Proskauer and others, and the details appear to be derivative and are confusing. It is not recognizable as to species. The name appears to be validly published.

No.117. Bacillus janthinus Flügge, 1886, p.291.
(Bacillus janthinus Trevisan, 1889, p.18; Lustig, 1890, p.89 in part; Kruse in Flügge, 1896, Vol.2, p.312; Godfrin, 1934, p.95, 234 in part. Bacillus Janthinus Waeldele, 1938, p.40 in part. Bacillus ianthinus Lustig, 1893, p.76 in part. Bacillus violaceus de Toni and Trevisan, in Saccardo, 1889, p.978 in part. Bacterium jochromum de Lagerheim, 1891, p.77 in part. Chromobacterium ianthinum Breed et al., 1948, p.232 in part. Not Chromobacterium ianthinum Gilman, 1953, p.48, see No.59. ? Violetter bacillus Hueppe, 1884, p.375). Greek, violet-coloured, Latinized.

Flügge described a strain isolated by Hueppe at Göttingen which he considered to be Zopf's Bacterium ianthinum (No.115). This may be the same strain as that described by Hueppe in 1884 (No.141). The description is brief. It was a bacillus which produced colonies on gelatin which were at first white and later violet, beginning at the edges of the colonies. It grew on potato, and coagulated milk, which later became ammoniacal and peptonized, and deep blue. This was probably a Chromobacterium but of uncertain species. The name is validly published; the epithet is an orthographic variant of Zopf's.

No.118. Bacillus janthinus Jordan, 1890, p.840, Pl.IV, figs.12a, 12b, 12c.
(Bacillus janthinus Miquel and Cambier, 1902, p.689; Sternberg, 1893, p.631.) Greek, violet-coloured, Latinized.

Jordan isolated this from sewage effluent and identified it with Zopf's bacillus (No.115). It was a nonsporing motile rod showing barred staining, slowly liquefying gelatin, turning milk alkaline, and giving a violet growth on potato. It was apparently strictly aerobic, and did not grow well at 37°. It reduced nitrates rapidly and made milk alkaline. It is most likely to have been a psychrophil, as the growth on agar was probably membrane-like. The name is validly published.

No.119. Bacillus janthinus Tils, 1890, p.311.
(Bacillus violaceus de Lagerheim, 1891, p.77 in part.)
Greek, violet-coloured, Latinized.

This strain was from water. It was said to form spores and to liquefy gelatin, but few other details are mentioned except that it formed a violet pigment. It was presumably a strain of Chromobacterium but the species is uncertain. The name appears to be validly published.

No.120. Bacillus janthinus Voges, 1893, p.312.
Greek, violet-coloured, Latinized.

This was found in water at Kiel. It was a Gram-negative nonsporing rod, described as having two or three flagella at one pole, but long rods had many flagella arising from the sides. It was a strict aerobe, and scarcely grew at 37°. The pigment was clearly violacein. On gelatin the colonies were violet, and the medium was liquefied. On potato the growth was violet. It was not pathogenic for mice when injected. This strain was probably a psychrophilic strain, but this is not certain. The name is validly published.

No.121. Bacillus janthinus Wright, 1895, p. 450.
(Chromobacterium janthinum Ford, 1927, p.474).
Greek, violet-coloured, Latinized.

Wright considered that this organism, isolated from the Schuylkill River and from water at New York, was Zopf's bacillus (No.115). It was a medium sized rod, motile by a single polar flagellum, which did not form spores. It produced in gelatin stab an early liquefaction of napiform shape and grew in the depths of the medium. On agar and potato it produced a violet membranous growth, on broth it produced a violet membrane, and in litmus milk it gave alkalinity, bleaching of the litmus, but no clot. It did not grow at 35° to 36°.

This appears to have been a strain of Chromobacterium, but the species is uncertain, as the behaviour in gelatin stab conflicts with the rest of the description. The name is validly published.

No.122. Pseudomonas pseudoviolacea Migula, 1900, p.943.
(Pseudomonas pseudoviolacea Migula, 1901, p.383; Breed et al., 1948, p.234; Krassilnikov, 1949, p.386. Bacillus pseudoviolaceus Matzuschita, 1902, p.138; Calderini, 1925, p. 768. Bacterium pseudoviolaceum Enderlein, 1925, p. 281.) Greek, false, and Latin, violet-coloured; Modern Latin adj., false violet-coloured.

This strain was isolated from water of the River Rhine. It was a motile rod, measuring 0.4 x 3-4 μ with a single polar flagellum. It showed barred staining and formed no spores. On agar it gave a violet growth, later becoming almost black, and the medium was stained a greenish colour. It grew sparingly in the depths of a gelatin stab and quickly liquefied the medium. This may have been a mesophilic strain, but this is uncertain. The name is validly published.

No.123. Bacteridium violaceum Schroeter, 1872, pp.124,126.
(Micrococcus violaceus Cohn, 1872, p.157; Winter in Rabenhorst, 1881,

p.44; Flügge, 1883, p.100; Grove, 1884, p.9; Crookshank, 1887, p.219; Cornil and Babes, 1890, Vol.1, p.146, fig.38; Lustig, 1890, p.61; 1893, p.36; Roux, 1892, p.289; Bergonzini, 1892, p.200; Sternberg, 1893, p.601; Frankland and Frankland, 1894, p.495; Migula, 1900, p.186; 1901, p.128; Miquel and Cambier, 1902, p.666; Calderini, 1925, p.773; Ford, 1927, p.434. Mikrococcus violaceus Adametz, 1888, p.34; Matzuschita, 1902, p.435. Mikrokokkus violaceus Frosch and Kolle in Flügge, 1896, Vol.2, p.181; Eisenberg, 1891, p.42. Cromococcus violaceus Bergonzini, 1879, p.37; 1880, p.150. Pseudomonas violacea Krassilnikov, 1949, p.386. Streptococcus violaceus Trevisan, 1889, p.31; de Toni and Trevisan in Saccardo, 1889, p.1067; Chester, 1901, p.70. Bacterium violaceum de Lagerheim, 1891, p.77 in part. Bacillus violaceus de Toni and Trevisan in Saccardo, 1889, p.978 in part; Godfrin, 1934, pp.85, 229 in part; Waeldele, 1938, p.33. Chromobacterium violaceum Bergey et al., 1934, p.170; 1939, p.92; Breed et al., 1948, p.231; Breed, Eltinge and Tobie in Breed et al., 1957, p.294. ? Violetter coccus, Maschek, 1887, p.68. ? Bacterium violaceum Schroeter, 1886, p.157, see No.126. ? Bacterium violaceum Lehmann and Neumann, 1899, Vol.1, Taf.23, Vol.2, p.262; 1920, Vol.1, Taf.31, Vol.2, p.403; 1927, Vol.1, Taf.23, Vol.2, p.463, see No.78; Enderlein, 1925, p.281; Cruess-Callaghan and Gorman, 1935, p.216. ? Pseudomonas violacea Pribram, 1933, p.49. ? Chromobacterium violaceum Leifson, 1956b, p.399, see No.109. ? Chromobacterium violaceum Eltinge, 1957, p.41, see No.110. ? Chromobacterium violaceum Hauduroy et al., 1937, p.88; Prévot, 1948, p.16. Not Chromobacterium violaceum Bergonzini, see No.124). Latin adj., violet-coloured.

The description of this organism, the first member of the genus to be discovered, is very brief. It was found growing on potato. The description may be translated as follows (Schroeter, 1872, p.124):

"Violet Organisms. One of the most beautiful of the chromogens mentioned here was one I saw in January 1870 on slices of potato which Dr. Schneider had left exposed to the air for bacterial and fungal growth. Besides a little mass of B. prodigiosus and of the yellow droplets mentioned above, there was a small shiny lump of a vivid violet colour. It grew and spread on the surface as a fleck until it was perhaps 6 mm in diameter. The mass consisted of nonmotile colourless elliptical cells, bigger than B. prodigiosus and different in that it was in chains of several members. I did not find any other cultures, and the pigment was not investigated more closely."

Cohn (1872, p.157) redescribed it (as Micrococcus violaceus) without adding more details, and there was general uncertainty among contemporary authors as to its identity. Clearly it cannot be recognized from this description, and only by tradition is it included in the genus Chromobacterium. Most other information is from organisms of doubtful authenticity isolated by subsequent workers (see Migula, 1900, p.186; Bergonzini, 1892, p.200; Lustig, 1890, p.61; 1893, p.36; Cornil and Babes, 1890, p.146 and fig.38). The name is validly published.

No.124. Cromobacterium violaceum Bergonzini, 1880, p.153. (Cromobacteriun [sic] violaceum Bergonzini, 1880, p.153. [Parte scientifica]. Cromobacterium [sic] violaceum Bergonzini, 1880, p.37 Parte officiale; Zimmermann, 1880, p.1529. Chromobacterium violaceum Anonymous, 1881, p.285; Buchanan, 1918, p.52; 1925, p.258; Holland, 1920, p.222; Winslow et al., 1920, p.209; Enlows, 1920, p.28; Bergey et al., 1923, p.118; 1926, p.123; 1930, p.158; 1934, p.170 in part; 1939, p.92 in part; Breed et al., 1948, p.231 in part; Breed, Eltinge and Tobie in Breed et al., 1957, p.294; Ford, 1927, p.469; Topley and Wilson, 1929, Vol.1, p.401; 1936, p.496; Wilson and Miles, 1946, Vol.1, p.634; 1955, Vol.1, p.729; Godfrin, 1934, pp.88, 230; Waeldele, 1938, p.36; Krassilnikov, 1949, p.501; Eltinge, 1957, p.43, see No.110. Bacterium violaceum Grove, 1884, p.26; Trelease, 1885, p.205; Pl.XII, fig.9, see No.125; de Lagerheim, 1891, p.77 in part. Bacillus violaceus de Toni and Trevisan, 1889, p.978 in part. ? Chromobacterium violaceum Hauduroy et al., 1937, p.88; Prévot, 1948, p.16. ? Chromobacterium violaceum Leifson, 1952b, p.399, see No.109. ? Chromobacterium violaceum Eltinge, 1957, p.41, see No.110. Not Cromococcus violaceus (Schroeter) Bergonzini, see No.123.) Latin adj., violet-coloured.

This organism was discovered and described by Bergonzini (1880) and placed by him in his subgenus Cromobacterium Bergonzini 1879, p.38. The spelling of the subgeneric name in 1880 shows a misprint (a terminal n) but it is correctly printed on page 37 of the Parte officiale with a final m. The preferable transliteration of the Greek gives the form Chromobacterium, first used in a review in the Journal of the Royal Microscopical Society (Anonymous, 1881).

Bergonzini made it quite clear that his organism was not the same as Bacteridium violaceum Schroeter 1872 (No.123) which Bergonzini called Cromococcus violaceus (Bergonzini, 1879, p.37; 1880, p.150). He regarded his own bacterium as a new species. A number of authors have confused these two, and cite Chromobacterium violaceum (Schroeter) Bergonzini which is incorrect, and do not mention whether they mean Bacteridium violaceum Schroeter 1872 (No.123), Bacterium violaceum Schroeter, 1886, (No.126) or Cromobacterium violaceum Bergonzini, 1880, or some or all of these.

The organism was found on 26th April 1880 forming a thick violet pellicle on a solution of white of egg which was exposed to the air and had evaporated to half its original volume. The fluid beneath was no longer opalescent, but was yellowish-green. The pellicle consisted of a zoogloea composed of rod-shaped organisms which were nonmotile, but organisms which were free in the fluid were described as motile, having an oscillating movement. The organisms were arranged singly. Single organisms appeared colourless, but in the mass they were violet. Bergonzini's diagnosis (1880, p.153) was:

"Cromobacteriun violaceum - Elementi cellulari cilindrici per lo piu isolati,2 a 3 volte cosi lunghi come larghi dotati di movimento oscillante, colorate in violetto da una sostanza speciale insolubile nell'acqua, - Grossezza da 0.6 a 1 μ; lungheza da 2 a 3 μ. Vegatano specialmente nelle soluz. d'albume d'ovo." The name was validly published.

The few other details which Bergonzini gave are important, for they show that the pigment was almost certainly violacein and the organism was therefore almost certainly a strain of Chromobacterium; it is the type species of Chromobacterium Bergonzini and thus occupies a central position in the nomenclature. The pigment in the zoogloea was insoluble in water, very soluble in alcohol giving a violet-blue solution, and slightly soluble in ether giving a reddish-violet solution. On diluting alcoholic solutions with water the pigment remained in solution; on adding hydrochloric acid they became green; on adding nitrohydrochloric acid (presumably aqua regia) they became yellow and did not become violet again when neutralized. Alcoholic solutions became cloudy on adding ammonia and a violet-grey flocculent precipitate formed, leaving a colourless supernatant. Solutions of pigment gave an amorphous residue on evaporation. On soaking the pellicle in strong caustic soda it slowly became decolorized and the liquid was slightly reddish. The alcoholic solution showed absorption of yellow and orange light when examined in a spectroscope, and there was also a slight absorption line between the C and D Frauenhofer lines (i.e. at about 620 mμ). These findings are in good agreement with the properties of violacein (see Chapter X).

Bergonzini was able to subculture the organism in white-of-egg solutions, but sometimes the pellicle was violet only in patches. The pellicle usually appeared after two weeks at room temperature and he also obtained violet patches on inoculating a solution of white-of-egg which was already putrefying. He was unable to obtain subcultures on milk or other media. It is clear that Bergonzini was probably not studying a pure culture, since it was probably contaminated with organisms from the air and from water. He was at this time exposing solutions of white-of-egg to the air during experiments on putrefaction, and he did not mention that the water had been sterilized. The subcultures which were violet in patches are suggestive of contamination. Some experiments of my own reinforce this belief, since in pure culture none of my strains of Chromobacterium formed pellicles of the kind described by Bergonzini. These experiments are described below.

The white of one hen's egg (opened with aseptic technique) was mixed with 300 ml of sterile distilled water, and 5 ml amounts were distributed into sterile plugged tubes of 3 x 20 cm size. The tubes were inoculated with thirty-eight strains of Chromobacterium in Table 1, Chapter III (twenty strains of mesophils and eighteen strains of psychrophils). They were incubated at room temperature, which was close to the temperature used by Bergonzini viz. 16° to 20°. None of the strains grew well, the majority showing only faint turbidity. Even after 6 months, when the tubes had almost dried up, none of the strains had produced a pellicle. It did not seem feasible to study the growth on contaminated medium, since the type of contaminant would obviously affect the results. At the temperature used by Bergonzini the rate of growth of mesophils and of psychrophils is about the same, and the size of the cells does not enable one to decide which type his strain was. The species of Bergonzini's organism is therefore unrecognizable, and being based on a mixed culture, the name is strictly not valid, but if the generic name Chromobacterium is conserved, it is also necessary to conserve a recognizable

type species. I therefore included in my proposals to the Judicial Commission of the International Committee on Bacteriological Nomenclature (Sneath, 1956a) the proposal that Bergonzini's organism be accepted as type species, but redefined by establishing it as the mesophilic species with type strain MK (NCTC 9757, ATCC 12472, see also p. 337), and this the Commission has approved (Judicial Commission, 1958a; 1958b; and see Chapter I).

Biographical Note. Curzio Bergonzini was born at Modena in 1851 and died in 1896. He was a Professor in the University at Modena and published in a wide field of scientific work. An obituary and list of publications is given by Picaglia (1896).

No. 125. Bacterium violaceum Trelease, 1885, p. 205, Pl. XII, fig. 9. (Bacillus violaceus de Lagerheim, 1891, p. 77). Latin adj., violet-coloured.

Trelease was sent this organism by W.G. Farlow. It formed a violet waxy growth on potato, composed of rods measuring 0.3 to 0.4 by 0.6 to 1.6 μ, which may have had polar granules. It was very easily killed by light. It was not examined on other media. The pigment was violet but is not further described. From these scanty details the organism seems to have been probably a strain of Chromobacterium, but of uncertain species. The name was validly published, but the date was not given in the exemplar I have seen: de Lagerheim gives it as 1885.

No. 126. Bacterium violaceum Schroeter, 1886, p. 157. (Bacterium violaceum de Lagerheim, 1891, p. 77 in part. Bacillus violaceus de Toni and Trevisan in Saccardo, 1889, p. 978; Macé 1889, p. 525; 1897, p. 853; 1913, Vol. 2, p. 412 in part; Godfrin, 1934, pp. 85, 229 in part; Waeldele, 1938, p. 33. Bactrinium violaceum Fischer, 1903, p. 148. Pseudomonas violacea Migula 1895, p. 29; 1900, p. 939 in part; 1901, p. 378; Holland, 1920, p. 221; Enlows, 1920, p. 74. Chromobacterium violaceum Bergey et al., 1934, p. 170 in part; 1939, p. 92 in part; Breed et al., 1948, p. 231 in part. ? Bacterium violaceum Lehmann and Neumann, 1899, Vol. 1, Tab. 23, Vol. 2, p. 262; 1920, Vol. 1, Taf. 31, Vol. 2, p. 403; 1927, Vol. 1, Taf. 23, Vol. 2, p. 463 (No. 78); Enderlein, 1925, p. 281; Cruess-Callaghan and Gorman, 1935, p. 216 (No. 134). ? Bacillus violaeus Miquel and Cambier, 1902, p. 687, in part. ? Pseudomonas violacea Pribram, 1933, p. 49. ? Chromobacterium violaceum Leifson, 1956b, p. 399 (No. 109); Eltinge, 1957, p. 41 (No. 110); Hauduroy et al.; 1937, p. 88; Prévot, 1948, p. 16.) Latin adj., violet-coloured.

Schroeter (1886) did not make it clear whether this organism is the same as his own Bacteridium violaceum Schroeter 1872 (No. 123). He cites it as synonymous with Bacillus violaceus Schroeter 1880 and with Bacteridium violaceum and Bacteridium ianthinum Zopf, but have not been able to discover Schroeter's description of 1880. It appears, however, that he intended it to include all the violet bacteria of which he knew, and is therefore described separately here.

Schroeter's description is as follows:

"Bacterium violaceum (Bacillus violaceus Schroeter, 1880, Bacteridium violaceum, Bacteridium ianthinum Zopf).

Zellen anfangs elliptischen, oft zu Längeren Ketten verbunden, Später stäbchenförmig, etwa 0.6-1.0 μ breit, 3-5 μ lang. Bildet dicke schön veilchenfarbene Schleimüberzüge. Farbstoff in Wasser unlöslich, in Alkohol leicht löslich mit schönvioletter Farbe: Eissigsäure und verdünte Mineralsäure verändern den Farbstoff nicht, Ammoniak und Aetzkali bringen eine grünlich Färbung hervor. Auf gekochten Kartoffeln und anderen Vegetabilien. Im Winter-Breslau nicht häufig beobachtet."

This description is scarcely more detailed than that of 1872 (see No. 123); it is probably Chromobacterium but of uncertain species. The name is validly published.

No.127. Bacillus violaceus Adametz, 1888, p.59. Taf.VI, fig.3, 4, Taf.V, fig.2.1. Latin adj., violet-coloured.

This organism was from water. It was cited as Bacillus violaceus Zopf. It was a motile rod, of dimensions 0.5 x 2 μ, sometimes showing filamentous forms. It was said to form central spores. The colonies became violet first at the edge. Gelatin was slowly liquefied. It gave a violet growth on potato, poor growth in "sugar with salts," blue flecks in milk, which became alkaline and in which the casein was precipitated. The species is uncertain. The name may be invalid since Adametz did not consistently use Linnaean binomials.

No.128. Bacillus violaceus Macé, 1889, pp. 525, 688. (Bacille violet Macé, 1887, p.354. Bacille violaceo Lustig, 1890, p.89. Bacillus violaceus Macé, 1897, p.853; Lustig, 1893, p.75 in part. Bacillus violaceus Lutetiensis Kruse in Flügge, 1896, p.311; Matzuschita, 1902, p.138; Calderini, 1925, p.767; Breed et al., 1948, p.233. Bacillus Lutetiensis Chester, 1901, p.306. Pseudomonas janthina Chester, 1901, p.317 in part. Chromobacterium violaceum lutetiense Ford, 1927, p.470. Bacillus Violaceus Lutetiensis Horrocks, 1901, p.69. ?Bacillus violaceus Macé, 1898, p.56, Pl.28; 1901, p.918; 1913, Vol.2, p.412; Miquel and Cambier, 1902, p.687 in part; Godfrin, 1934, pp.85, 229 in part; Waeldele, 1938, p.33). Latin adj., violet-coloured.

Macé (1887) described this organism as a new form. It was isolated from water, and consisted of rods measuring about 0.5 to 0.7 by 1.5 to 2 μ. It formed no spores and rapidly liquefied gelatin. Colonies on agar became violet first at the edge. It grew on potato. Cultures smelt of cream cheese, and later of butyric acid. It grew slowly at room temperature. This was presumably a strain of Chromobacterium but the species is uncertain. It is probably the organism referred to by many French authors as Bacillus violaceus Macé when they do not cite the reference fu Bacillus violaceus Macé 1889 is validly published, as is Bacillus Lutetiensis Chester 1901. Kruse's name is an invalid trinomial. The specific epithet 'lutetiensis' is from the Latin name for the city of Paris; in fact the organism was isolated at Sézanne.

No.129. Bacillus violaceus Frankland and Frankland, 1889, p.394. Taf. IV, figs. 1A to 1E.
(Bacillus violaceus Jordan, 1890, p.842; Lustig, 1890, p.88; 1893, p.75 in part; de Lagerheim, 1891, p.77 in part; Roux, 1892, p.307; Sternberg, 1893, p.641; Voges, 1893, p.310; Miquel and Cambier, 1902, p.687 in part; Matzuschita, 1902, p.50 in part; Godfrin, 1934, pp.85, 229 in part; Waeldele, 1938, p.33; Breed et al., 1948, p.233. Bacillus violaceus Berolinensis Kruse in Flügge, 1896, Vol.2, p.311 in part. Bacillus Violaceus Berolinensis Horrocks, 1901, p.68 in part. Bacillus janthinus Frankland and Frankland, 1894, p.472. Pseudomonas pseudianthina Migula, 1900, p.942, Tab.XIII, fig.6; 1901, p.382. Pseudomonas janthina Chester, 1901, p.317 in part).
Latin adj., violet-coloured.

This organism was isolated by the Franklands from water of the Rivers Thames, Lee, and Spree. It was a rod measuring 0.8 μ by 1.7 μ approximately, motile, and said to have central spores (probably an artifact). It produced violet colonies on gelatin, which was slowly liquefied. It grew poorly on potato, and reduced nitrate to nitrite. Migula (1900) found a single polar flagellum. This was a strain of Chromobacterium of uncertain species, though it was possibly a psychrophil. Frankland and Frankland's name was validly published in 1889, as was Migula's name in 1900.

No.130. Bacillus violaceus Zimmermann, 1890, pp.34(82), 92(140).
(Bacillus violaceus Schneider, 1894, p.227; Matzuschita, 1902, p.50 in part. Pseudomonas violacea Migula, 1900, p.939 in part.)
Latin adj., violet-coloured.

This organism was isolated from water at Chenmitz. It was a rod measuring 0.65 μ by 1 to 3 μ, motile and Gram-negative. It was said to form spores. It liquefied gelatin, gave a violet growth on potato, produced turbidity without a pellicle in broth, was strictly aerobic and its optimum temperature was room temperature. The violet pigment was investigated by Schneider, and seems certainly to have been violacein. This organism was a strain of Chromobacterium but the species is doubtful. The name was validly published.

No. 131. Bacillus violaceus Voges, 1893, p. 310.
Latin adj., violet-coloured.

This organism was identified by Voges with Bacillus violaceus Frankland 1889 (No.129). It was a Gram-negative rod, motile by a single polar flagellum, and did not form spores. It liquefied gelatin within a week, but did not grow at 37° and was strictly aerobic. The violet pigment had the properties of violacein. It was a strain of Chromobacterium but is of uncertain species. The name is validly published.

No.132. Bacterium violaceum Wolff, 1911, p. 643.
Latin adj., violet-coloured.

This strain was isolated from water. It was a motile nonsporing rod producing a violet growth on gelatin and potato. It liquefied gelatin and turned milk alkaline. It grew at 30° but produced little pigment at this temperature. Wolff cited it as a new species in the title of his paper, though he remarks that it was very similar to Lehmann and Neumann's Bacterium violaceum (No. 78) of which it is a later homonym. It was a strain of Chromobacterium but the species is uncertain.

No. 133. Bacillus violaceus Mehta, 1925, p. 342.
Latin adj., violet-coloured.

This strain was NCTC 1539, which is unfortunately now lost. It was a Gram-negative nonsporing rod without a capsule, motile by several peritrichous flagella. It produced on agar and gelatin colonies which were at first yellowish and later violet. It liquefied gelatin, and was facultatively anaerobic. Milk was not peptonized or coagulated. It gave a mixed violet and yellow growth on potato, and a pellicle on peptone water. It was indole-negative, reduced nitrate to nitrite, and produced no acid from carbohydrates. This was presumably a strain of Chromobacterium but the species is uncertain. The name is validly published.

No. 134. Bacterium violaceum Cruess-Callaghan and Gorman, 1935, pp. 216-217, Pl. 6, figs. 5, 7.
(Chromobacterium violaceum Bergey et al., 1939, p. 94; Breed et al., 1948, p. 231; Breed, Eltinge and Tobie in Breed et al., 1957, pp. 292, 294). Latin adj., violet-coloured.

Eleven strains were placed in this species, which was identified with Bacterium violaceum Schroeter but whether No. 123 or No. 126 was not stated. They were Gram-negative rods, 0.4-0.6 x 1.8-3.5 μ, motile by a single polar flagellum. Little or no growth occurred on potato; on agar it was deep violet. Gelatin was rapidly liquefied, but there was little peptonization of milk. They were indole-negative, reduced nitrate to nitrite, gave acid without gas in glucose, usually in maltose, not in sucrose. They showed slight growth at 4° but none at 37°. The name is validly published. The species is uncertain; perhaps both mesophilic and psychrophilic strains were included.

No. 135. Bacillus violaceus laurentius Dyar, 1895, p. 372.
Latin adj., violet-coloured, and Modern Latin adj., pertaining to the town of Lawrence, Massachusetts.

This was a nonsporing motile rod, which produced a violet pigment. It liquefied gelatin rapidly, clotted milk, and rapidly reduced nitrates. It grew poorly on potato. Probably a Chromobacterium but of uncertain species: Dyar regarded it as the same as Bacillus violaceus, so his name may not be legitimate.

No. 136. Bacillus violaceus Sartoryi Waeldele, 1938, p. 55.
(Bacillus violaceus sartoryi Breed et al., 1948, p. 233.) Latin adj., violet-coloured, and Modern Latin, of Sartory (for Prof. A. Sartory).

This organism was described by Sartory, Meyer and Waeldele (1938a; 1938b) and named by Waeldele (1938). It was isolated from dental pus, but was thought to have been a contaminant (Waeldele, 1938, p. 55). It was a short rod, often coccobacillary, motile by a single polar flagellum, and Gram-negative. It was said to form spores, but these were only observed by examining stained preparations, and their heat resistance was not tested: they were probably artifacts. It was a strict aerobe, which grew at 8° and at 32° but not at 37°; the optimum temperature for growth was about 27°. It liquefied gelatin rapidly, liquefied coagulated serum slowly and peptonized milk slowly. On broth it produced a thick pellicle. It was said to be indole-positive and H_2S-negative. All carbohydrates tested were attacked without the production of gas or of appreciable amounts of acid.

Upon agar it produced a violet pigment with chemical properties in good agreement with that of violacein, although as Beer et al., (1949) point out, there are some discrepancies (see Chapter X). The absorption spectrum of the pigment was in good agreement with that of violacein. This strain was probably a strain of Chromobacterium, but its species is uncertain. The description would fit a psychrophilic strain better than a mesophil. The name is an illegitimate trinomial; it is not a subspecific ternary name, since Waeldele (1938, pp. 21, 55) considered it a new species, Bacillus violaceus N.Sp. Sartoryi [sic].

No.137. Bacteria violada de Lagerheim, 1891, p. 74.

This was found growing on potato on which it formed a touch mucilaginous violet growth, composed of nonsporing rods. It may have belonged to Chromobacterium and was possibly a psychrophil though this is uncertain.

No.138. Group VI of Calderini (1925, p. 779).

These organisms from water were briefly described but not named. They were considered by Calderini to be different from the other violet chromogens he studied. They were polar flagellated, monotrichous, Gram-negative rods which gave a violet pigment on potato, but on agar produced a greenish fluorescent growth. Gelatin was liquefied, glucose was fermented with production of acid without gas and on broth a pellicle was formed. They were indole-positive. The violet pigment resembled violacein in being soluble in alcohol but not in water or in chloroform and in becoming green with caustic potash.

It is unfortunate that the strains were not more fully described, for they are of interest in that they may have been intermediate forms between Chromobacterium and Pseudomonas. Strains of this type do not seem to have been isolated by other workers. They are included here as aberrant strains of Chromobacterium of uncertain species.

No.139. Group VII of Calderini (1925, p. 779).

This group was not named by Calderini. They were nonmotile, Gram-negative rods producing a violet growth on gelatin, very little pigment on

agar, but on potato a tenacious violet membrane. They did not liquefy gelatin and grew poorly in broth. They were indole-negative. Milk was coagulated. The violet pigment behaved like violacein toward alkalis and solvents. They appear to be close to Eisenberg's Bacillus membranaceus amethystinus (No. 100) and were therefore probably psychrophils.

No. 140. Bacille violet, Bidet, 1900, cited by Miquel and Cambier, 1902, p. 697.
(Bacille violet de la Dhuis, Miquel and Cambier, 1902, p. 697.)

I have not seen the original description by Bidet. Miquel and Cambier report it as a motile nonsporing rod, growing at 20° but not at 37°, producing a violet pigment, and liquefying gelatin rapidly. In broth it produced a tough pale violet pellicle. The species is uncertain.

No. 141. Violetter bacillus, Hueppe, 1884, p. 365.
(? Bacillus janthinus Flügge, 1886, p. 219, see No. 17; Lustig, 1890, p. 89. ? Bacillus ianthinus Lustig, 1893, p. 76.)

This was a rod which grew on potato giving a violet or blue-black growth. It liquefied gelatin, and peptonized milk giving a blue-black pellicle. It was from water. It was probably a Chromobacterium but of uncertain species.

No. 142. Violetter Bacillus, Maschek, 1887, p. 71.

This was a motile rod which liquefied gelatin and produced a violet pigment after several days growth. It was isolated from water. Maschek identified it with Zopf's organism (No. 115). The species is unrecognizable, but it was probably a Chromobacterium.

V. CHECK LIST OF EPITHETS USED IN COMBINATION WITH THE GENERIC NAME CHROMOBACTERIUM

Some authors, notably Topley and Wilson (1929) and Krassilnikov (1949) have included in Chromobacterium the Gram-negative aerobic rods which produce red or yellow pigments. This check list includes all those which I have found. Since the main purpose is simply to record the epithets used with the generic name the full citations and references are not given and the author's citations are quoted as given (unless clearly incorrect when checked against easily-available sources). Those described in Appendix II are referred to by number. The others are mainly species of Serratia, Flavobacterium, Erwinia, and Cellulomonas and most are listed in Breed et al. (1948).

The spelling Cromobacterium is treated as an orthographic variant. Although they may have the same epithet, the taxa are listed separately if they are based on different types or substantially different descriptions.

C. acetylicum (Levine and Soppeland) Krassilnikov, 1949, p.489.
C. acidificum (Steinhaus) Krassilnikov, 1949, p.488.
C. aeruginosum (Schroeter) Bergonzini, 1879, p.40. See No.27.
C. agarlyticum Krassilnikov, 1949, p.486, syn. Agarbacterium aurantiacum Angst.
C. amethystinum Holland, 1920, pp.217, 222. See No.100.
C. amethystinum Bergey et al., 1939, p.94. See No.105.
C. amethystium Krassilnikov, 1949, p.501. See No.92.
C. amethystinum [sic] Bergey et al., 1923, p.121. See No.100.
C. amocontactum (ZoBell and Allen) Krassilnikov, 1949, p.498.
C. amylorubrum (Hefferan) Krassilnikov, 1949, p.483.
C. ananas (Serrano) Krassilnikov, 1949, p.494, syn. Erwinia ananas Serrano. var. anitritum. See C. janthinum anitritum (No.86) and C. violaceum anitritum (No. 86).
C. anolium (Duran-Reynals and Clausen, 1937, p.369) Krassilnikov, 1949, p.485.
C. antenniforme (Ravenel) Krassilnikov, 1949, p.491.
C. aquatilis (Frankland and Frankland) Topley and Wilson, 1929, Vol.1, p.404; syn. Flavobacterium aquatilis Bergey et al., 1923, p.100.
C. armeniaca (Panosjan and Mirsabekjan) Krassilnikov, 1949, p.494.
C. aromaticum (Pammel) Krassilnikov, 1949, p.488.
C. aurantiacum (? Bacillus aurentiacus Frankland and Frankland) Topley and Wilson, 1929, Vol.1, p.405. See also Krassilnikov, 1949, p.485.
C. aurantinum (Hammer) Krassilnikov, 1949, p.491.
C. aurescens (Ravenel) Krassilnikov, 1949, p.499.
C. aurogenes (Bergey et al., 1923, p.157) Krassilnikov, 1949, p.500.
C. balustinum (Harrison) Krassilnikov, 1949, p.490.
C. bamptonii Bergey et al., 1923, p.119. See No.92.
C. bauri (Parlandt) Krassilnikov, 1949, p.499.
C. biazoteum (Kellerman et al.) Krassilnikov, 1949, p.499.
C. brunneum (Schroeter) Bergonzini, 1879, p.40.
C. butyri (Bergey et al., 1923, p.106) Krassilnikov, 1949, p.491.
C. cassavae (Handsford) Krassilnikov, 1949, p.494.
C. chitinochroma (Hock) Krassilnikov, 1949, p.492.
C. chlorinum (Migula) Krassilnikov, 1949, p.487.
C. chlorum (Steinhaus) Krassilnikov, 1949, p.487.
C. chocolatum Lasseur et al., 1944b, p.293 ex Knutsen. See Nos.50, 51.
C. citricum (Kern) Krassilnikov, 1949, p.486.
C. coeruleum (Voges) Bergey et al., 1923, p.120. See No.48.
C. coeruleum Ford, 1927, p.475. See Nos.35 and 53.
C. cohaerens Grimes, 1930, p.383. See No.93.
C. cytolyticum (Chester) Krassilnikov, 1949, p.494.
C. denitrificans (Lehmann and Neumann) Topley and Wilson, 1929, Vol.1, p.405.
C. devorans (Zimmermann) Krassilnikov, 1949, p.499.
C. diffusum (Frankland and Frankland) Krassilnikov, 1949, p.487.
C. erivanense (Kalantarian) Krassilnikov, 1949, p.493.
C. esseyanum Hauduroy et al., 1953 Appendix p. [15] (nomen nudum).
C. ferrugineum (Rullmann) Krassilnikov, 1949, p.500.
C. flavescens (Pohl) Krassilnikov, 1949, p.497.
C. flavidum (Fawcett) Krassilnikov, 1949, p.495.

C. flavinum Krassilnikov, 1949, p.499, syn. Cellulomonas flava Sack.
C. flavoviridum (Migula) Krassilnikov, 1949, p.487.
C. flavum (Fuhrmann) Krassilnikov, 1949, p.498.
C. folium (Sanborn) Krassilnikov, 1949, p.500.
C. fuchsinum (Boekhout and De Vries) Krassilnikov, 1949, p.483.
C. fuscum (? Bacillus fuscus Zimmermann) Topley and Wilson, 1929, Vol.I, p.405.
C. galbum (Kellerman et al.) Krassilnikov, 1949, p.500.
C. gilvum (McBeth) Krassilnikov, 1949, p.500.
C. grani (Parlandt) Krassilnikov, 1949, p.487.
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C. ianthinum Breed et al., 1948, p.232. See Nos.115 and 117.
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C. leporis (Migula) Krassilnikov, 1949, p.491. Syn. Bacillus leporis lethalis Sternberg.
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C. manilae Leifson, 1956a, p.60; 1956b, p.399. See Nos.81 and 88.
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C. membranaceum amethystinum I, II, III and IV Ford, 1927, pp.472-474. See Nos.92, 99, 102, 103 and 104.
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var purpureum. See C. janthinum var purpureum (No.86) and C. violaceum var purpureum (No.86).
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C. radiatum Krassilnikov, 1949, p.486 syn. Bacillus radiatus aquatilis Copeland.
C. rheni (Chester, 1901, p.251) Krassilnikov, 1949, p.490.
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C. syncyanum (Ehrenberg) Bergonzini, 1879, p.39. See No.33.
C. synxanthum (Ehrenberg) Krassilnikov, 1949, p.490, syn. C. xanthinum Bergonzini.
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C. tremelloides (Tils) Krassilnikov, 1949, p.486.
C. typhi-flavum Topley and Wilson, 1938, p.500, fig.115.
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C. violaceum Hauduroy et al., 1937, p.88. See Nos.123,124 and 126.
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C. violaceum Breed et al., 1948, p.231. See Nos.78,123,126 and 134.
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C. violaceum Eltinge, 1957, p.41 and vars violaceum and mesophilum. See Nos.110,123,124 and 126.
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C. vitivorum (Baccarini) Krassilnikov, 1949, p.493.
C. xanthinum (Schroeter) Bergonzini, 1879, p.40, pro synon. Vibrio synxanthus Ehrenberg, 1840, p.202; syn. C. synxanthum Krassilnikov, see above.

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The work reported here was carried out in several institutions, given below. Most of the practical work was done by myself, but I had extensive technical assistance with the preparation of culture media, the serology, histological procedures and experiments with animals, and some of the work was done jointly with others. The substance of this monograph formed a dissertation for the degree of Doctor of Medicine, University of Cambridge, 25th September, 1958.

Places of work, appointments held and co-workers

Case 1 was seen at the Army Pathological Laboratory, British Military Hospital, Singapore, where I was a pathologist in the Royal Army Medical Corps.

Cases 2-4 were seen at British Medical Hospital, Kamunting, Taiping, Malaya, while I was Deputy Army Director of Pathology, Malaya District, at British Military Hospital, Kinrara, Kuala Lumpur, Malaya, and I collaborated in the investigations with Dr. J.P.F. Whelan and Dr. R. Bhagwan Singh of the Institute for Medical Research, Kuala Lumpur, and with Major H. D. G. Hetherington, R.A.M.C. and Major D. Edwards, R.A.M.C.

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laetando dicit!